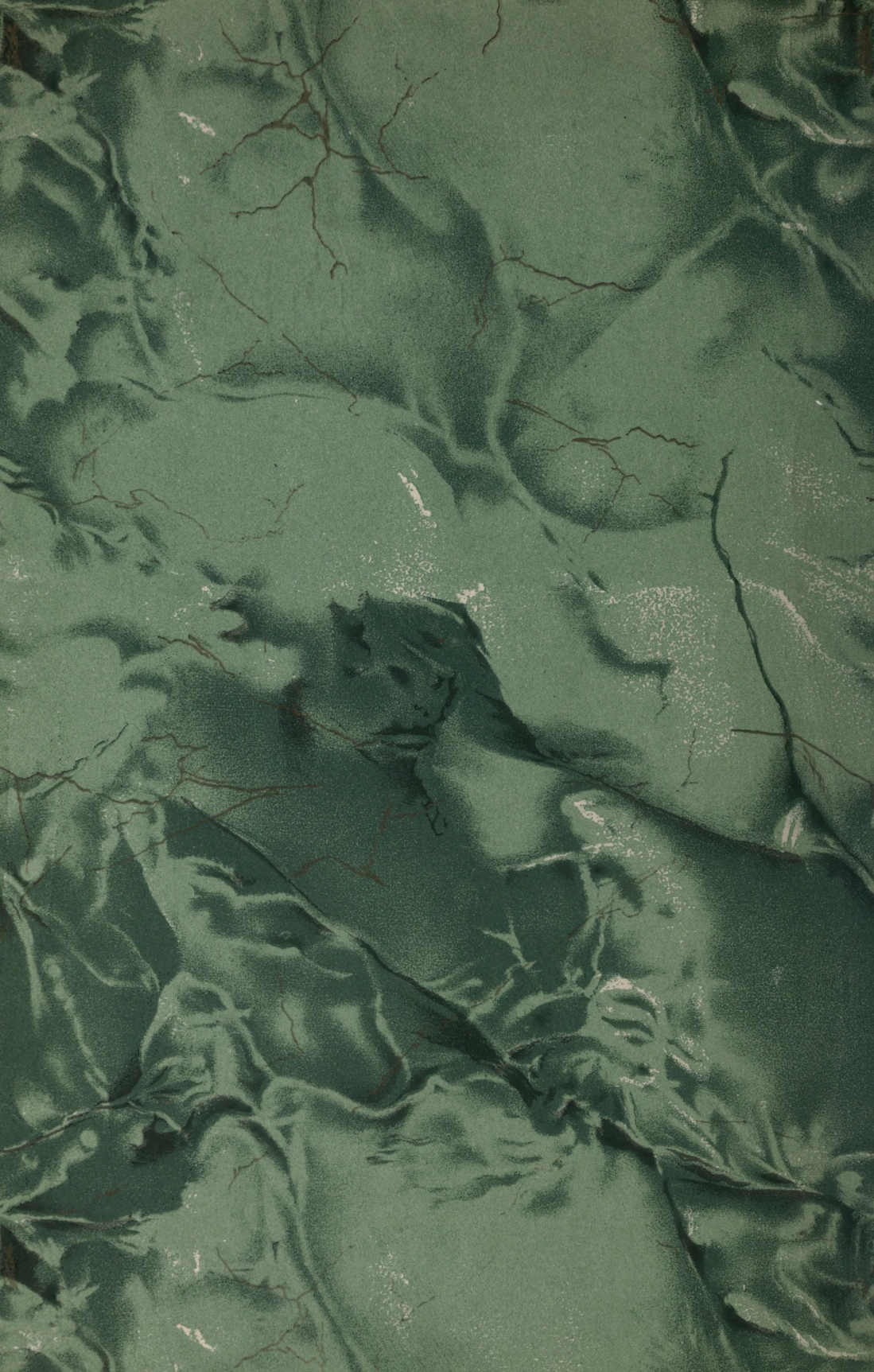

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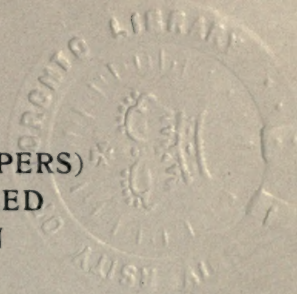

ANNUAL REPORT (REPRINTED PAPERS)
OF THE INVESTIGATIONS CARRIED
OUT UNDER THE SUPERVISION

OF THE

THERAPEUTIC RESEARCH COMMITTEE

OF THE

COUNCIL ON PHARMACY AND CHEMISTRY

OF THE

AMERICAN MEDICAL ASSOCIATION

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CONTENTS

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ON THE ABSORPTION OF LOCAL ANESTHETICS THROUGH THE GENITO-URINARY ORGANS*

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INTRODUCTORY

Local anesthetics are employed extensively in genito-urinary practice. They are administered for the performance of minor operations, such as circumcisions, urethrotomies, etc., and are instilled into the urethra and bladder to relieve pain and facilitate the introduction of instruments, such as sounds, endoscopes, cystoscopes, dilators, etc. In gynecological practice, also, local anesthetics are applied to the urethra and bladder for the same purposes; and occasionally cocain suppositories are introduced even into the vagina for the relief of itching or other irritating or painful conditions.

In connection with a comparative study of local anesthetics in relation to the genito-urinary organs, which was undertaken by the author at the suggestion of the Council on Pharmacy and Chemistry of the American Medical Association, the author inquired into the question of absorption of these substances through the genito-urinary organs. In the present paper it is proposed to report in brief the results of this investigation.

Inasmuch as the possibility of absorption of local anesthetics from the genito-urinary tract is of interest chiefly from the the toxicological point of view; that is, in reference to the question of whether these substances are absorbed in sufficient quantities to produce toxic symptoms, the experiments in this investigation were confined for the most part to the most important, as well as one of the most poisonous drugs belonging to this class, namely, cocain. The other local anesthetics which were investigated in this respect were alypin and apothesin. The comparative toxicity of local anesthetics has been studied by various authors and in particular has been gone into recently very carefully by Eggleston and Hatcher,[1] and from the work of the latter it is evi-

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* From the Pharmacological Laboratory of the Johns Hopkins University and the James Buchanan Brady Urological Institute.

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1. Eggleston and Hatcher: Jour. Pharmacol. & Exper. Therap. **13**:433, 1919.

dent that among the more toxic local anesthetics which are employed in genito-urinary work are the three substances just mentioned. For this reason the present experiments were made with only these drugs.

ABSORPTION FROM THE BLADDER AND URETHRA

Method.—In a communication on the absorption of apomorphin and morphin from the urethra and bladder,[2] the author called attention to the fact that those drugs were admirably suited for the study of absorption through unusual channels or portals of entry, on the one hand, and that they could conveniently be employed for the study of the comparative degree of absorption of drugs from the urethra and

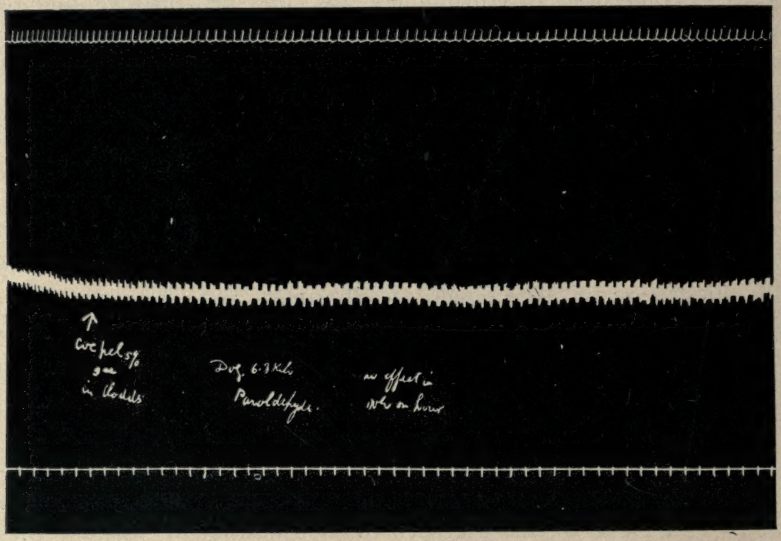

Fig. 1.—Dog, weighing 61.8 kg.; paraldehyd anesthesia. Five cubic centimeters of cocain hydrochlorid (5 per cent. solution) in bladder; no effect is shown on blood pressure or respiration after a period of over one hour.

bladder, on the other hand. In the present research, the same method of study was employed.

The study of absorption from bladder and urethra was confined to male animals. Dogs were found to be ideal subjects for this purpose, because, in the first place, the histologic structure of the urethra and bladder in the dog is practically the same as that in man, and secondly, because the vesical and urethral sphincters in this animal are so powerfully developed that it is possible to confine the action of a drug to the bladder or urethra at the pleasure of the experimenter.

2. Macht: J. Urol., **2**:43, 1918.

If a fine catheter, with a side opening and a wire guide, is passed through the urethra of a male dog into its bladder, and is left in position, the internal sphincter contracts so firmly around it that any fluid injected through the catheter into the bladder is practically confined to that cavity alone, and does not permeate into the urethra. Indeed, in some dogs it is almost impossible to pass a catheter into the bladder at all on account of the excessive irritability and spasmodic contraction of the sphincter muscles.

If, on the other hand, it is desired to limit the action of a drug to the urethral mucosa only and not the bladder, all that is necessary is to insert the catheter as far as the external sphincter, in which case

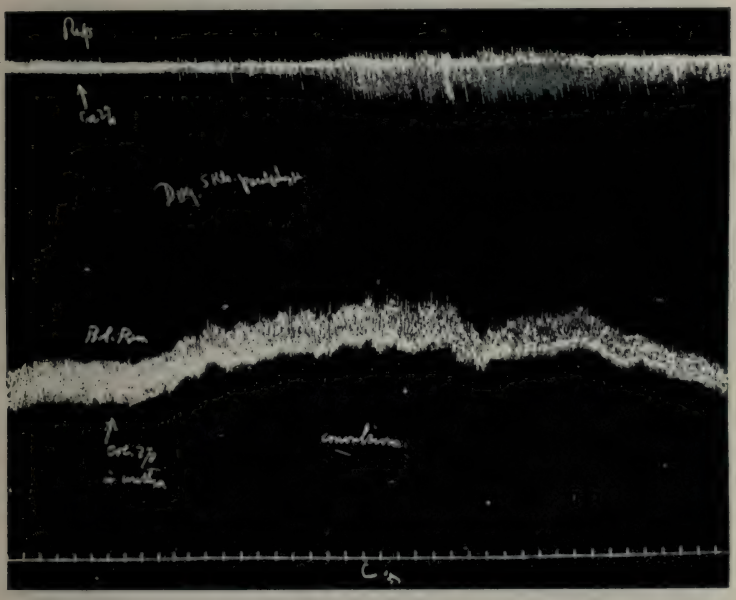

Fig. 2.—Dog, weighing 5 kg.; paraldehyd anesthesia. The effect of irrigating the urethra with a 2 per cent. solution of cocain hydrochlorid; note the rapid and marked effect on blood pressure and respiration.

a fluid injected through the catheter under moderate pressure will merely irrigate the urethra and flow out again externally without penetrating into the bladder. The conditions just described hold good for the great majority of dogs. In exceptional cases, of course, the sphincters may not be so well developed or many not contract so tightly, thus preventing a sharp differentiation in applying the drug to the bladder or urethra.

Action of Cocain.—The relative absorption of cocain from the bladder and urethra is strikingly illustrated by Figures 1 and 2. In

Figure 1, 5 c.c. of cocain hydrochlorid (5 per cent. solution) was introduced into the bladder of a dog and retained in the same by the method just described, for a period of over one hour. It will be noted that there was absolutely no effect produced on the respiratory and blood pressure curves. In Figure 2, the urethra of the same dog was irrigated slowly with a 2 per cent. solution of cocain hydrochlorid and very soon after the irrigation definite signs of absorption were manifested. The blood pressure was changed and the respiration was markedly stimulated, as it generally is at first by small doses of cocain. Following

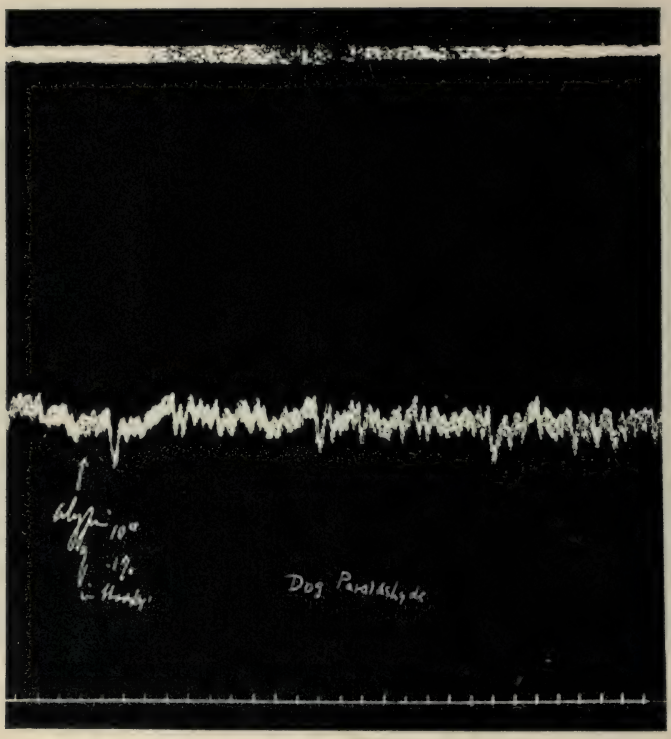

Fig. 3.—Effect of alypin on the absorption from the bladder. Ten cubic centimeters of a 0.1 per cent. solution produces no effect after one hour.

these symptoms, the dog developed convulsions, showing a deeper poisoning, and this was followed gradually by a fall in blood pressure and shallow respirations. It is thus evident that absorption of cocain from the urethra takes place very readily, whereas the same drug is but very poorly absorbed through the walls of the bladder.

Action of Alypin and Apothesin.—Experiments with alypin and apothesin (the hydrochlorid of diethyl-amino-propyl cinnamate) gave

results analogous to those obtained with cocain. These drugs were also much more readily absorbed from the urethra than from the bladder (Figs. 3 and 4).

ABSORPTION FROM THE URETER AND PELVIS OF THE KIDNEY

Method.—Absorption from the ureter and kidney pelvis was studied in dogs. In order to exclude the possibility of error through absorption through the peritoneum and other channels, the procedure was as follows:

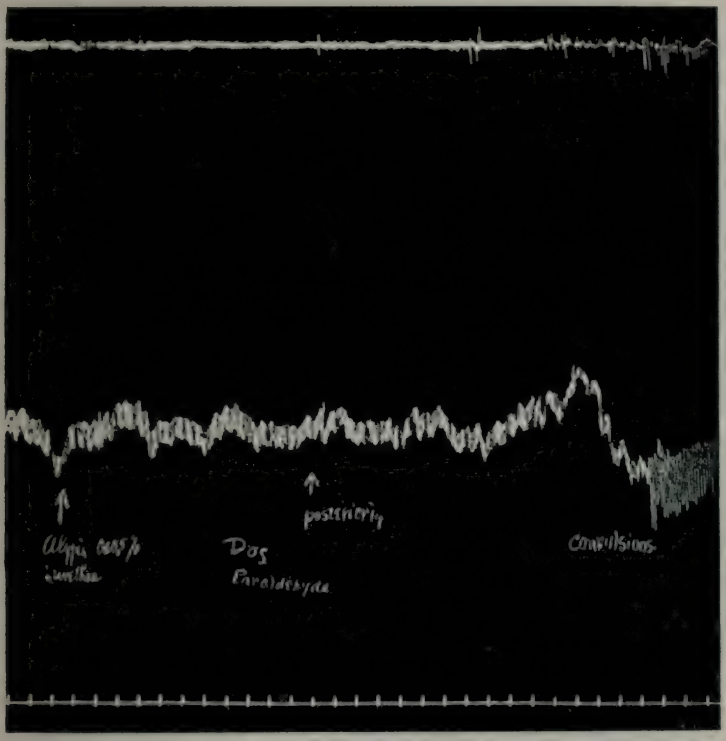

Fig. 4.—Absorption of alypin from urethra. Effect of irrigation with a 0.05 per cent. of the drug; upper curve, respiration; lower curve, blood pressure.

An incision was made just below the floating ribs and the kidney exposed. A glass cannula was then inserted into the ureter just below the kidney pelvis pointing distalward. A fine ureteral catheter was passed through the ureter from above downward and its insertion in the bladder located. An incision was then made in the abdominal wall just above the bladder and another cannula, with a small rubber tube attached, was inserted into the lower end of the ureter pointing upward. Particular pains were taken to ligate the cannulae tightly so as to pre-

vent leakage of the perfusing fluid. The whole length of the ureter was thus perfused with a saline solution by injecting the fluid under low pressure into the upper cannula and allowing the solution to escape from the lower cannula and rubber tubing without coming in contact with the peritoneum or entering the bladder. In this way normal physiological saline solutions and saline solution containing the drug to be studied could be perfused through the ureter alone and the absorption of drugs or poisons studied at ease.

In order to study absorption of drugs from the kidney pelvis, the procedure in the first steps was the same as above. An incision being made below the floating ribs, the kidney was exposed and its pelvis freed from surrounding structures. A good sized glass cannula was

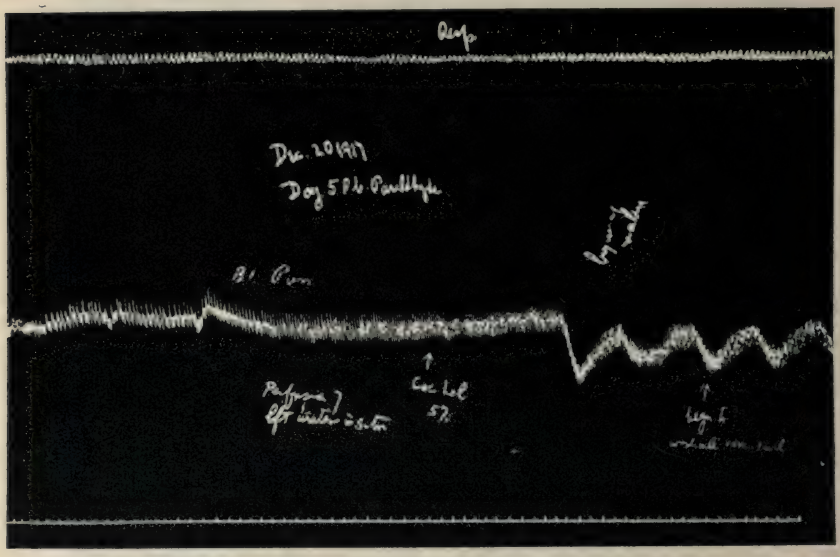

Fig. 5.—Absorption of cocain hydrochloride from urethra of the dog. Showing the effect of perfusion with a 1 per cent. solution.

then inserted into the pelvis and ligated pointing upward or toward the kidney. A fine ureteral catheter was then inserted loosely inside of the glass cannula and the perfusing fluid injected through the fine catheter, and allowed to flow back through the larger glass cannula surrounding it. A drug in solution could thus be brought in contact with the pelvis of the kidney under low pressure and the fluid allowed to run back around the catheter through the glass cannula without coming in contact with the peritoneum or surrounding tissues.

Effect of Cocain.—Experiments with cocain performed by the above method showed clearly that that drug is absorbed readily both through

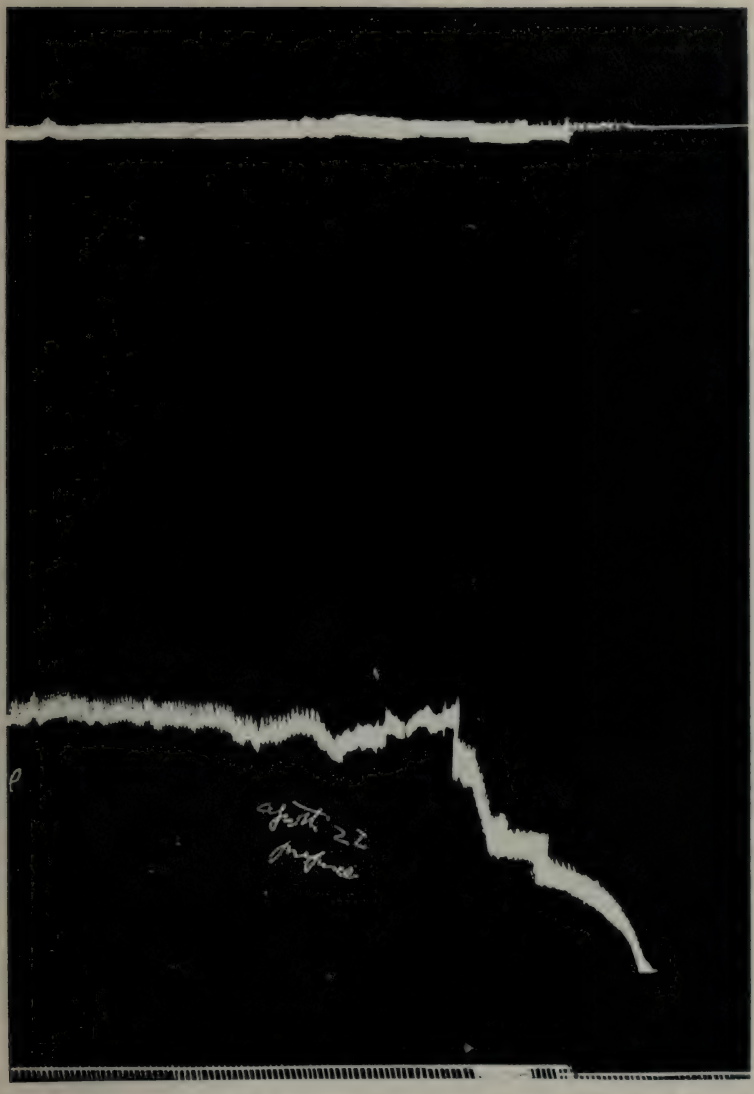

Fig. 6.—Dog, weighing 8 kg.; paraldehyd anesthesia. Effect of introducing two tablets of apothesin in the praeputial sac; upper curve, respiration; lower curve, blood pressure.

the ureteral walls and through the pelvis of the kidney. In Figure 5, the left ureter of a dog was perfused slowly with a 1 per cent. solution of cocain hydrochloride in physiological saline. The dog was anesthetized with paraldehyd and the respiratory and blood pressure curves were recorded. It will be seen that perfusion with cocain produced a fall in blood pressure, and convulsions, thus indicating that the drug was absorbed.

ABSORPTION THROUGH THE PRAEPUTIUM

A careful search of the literature by the author has failed to reveal any experiments bearing on the subject of absorption through the praeputium penis. An investigation was therefore begun by the author

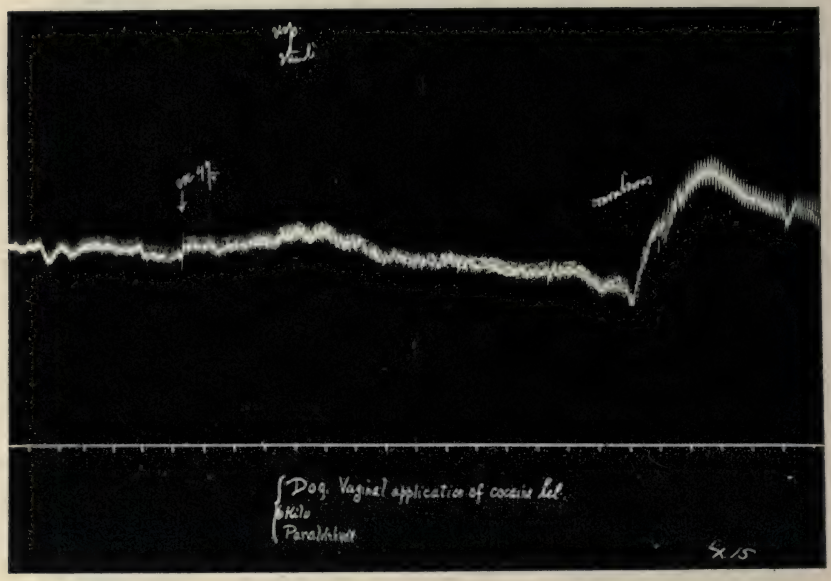

Fig. 7.—Dog, weighing 6 kg.; paraldehyd anesthesia. Five cubic centimeters of a 4 per cent. solution of cocain hydrochlorid was introduced into vagina. Note effect on blood pressure and convulsions. The respiratory curve is not shown in this figure.

concerning the absorption of different classes of drugs through that organ. In this place only the experiments with local anesthetics are recorded, while a fuller paper bearing on the subject in general will be published in the *Journal of Urology*.

For the purpose of experimentation, the dog is the most suitable animal, as the praeputium of that animal is highly developed. In the experiments performed, the drugs were introduced into the preputial sac and the physiologic effects pointing to their absorption into the gen-

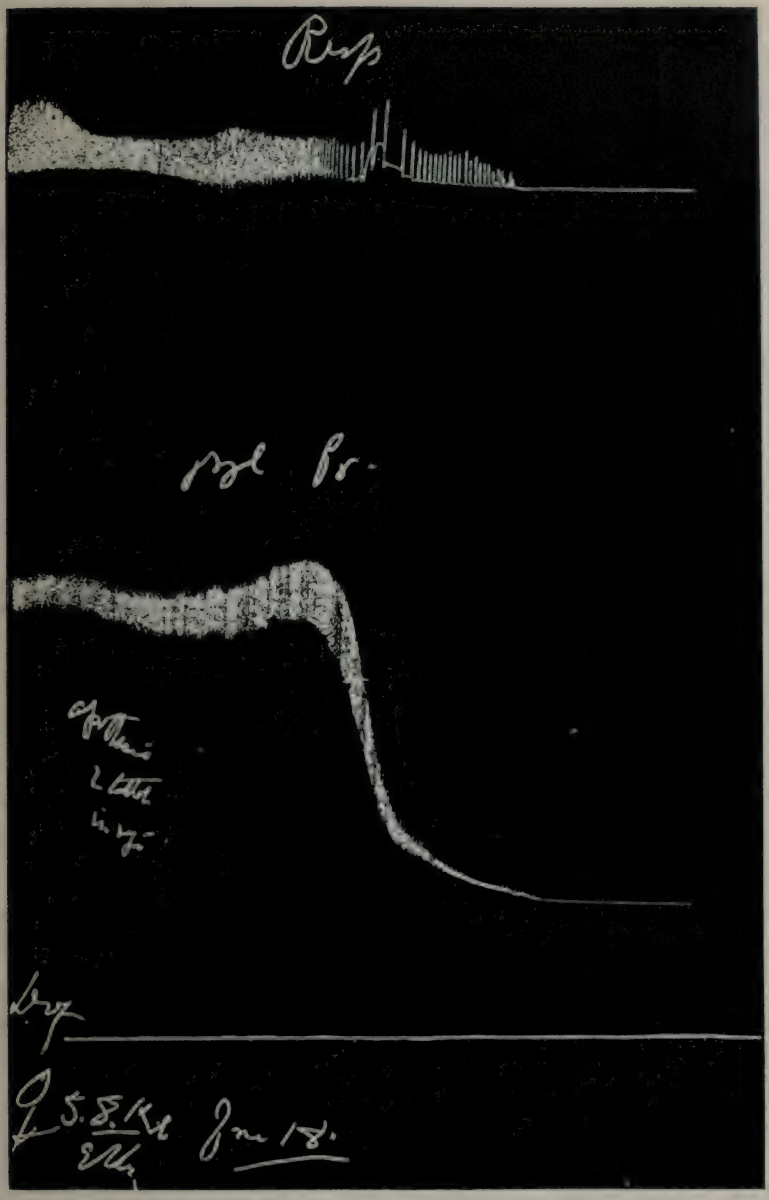

Fig. 8.—Dog, weighing 5.8 kg.; ether anesthesia. Effect which followed the introduction of two tablets of apothesin into the vagina.

Fig. 9.—Dog, weighing 10 kg.; paraldehyd anesthesia. At the points indicated by arrows, 4 c.c. of a 1 per cent. solution of apothesin was injected intravenously.

eral circulation were looked for. In case of the local anesthetics, the curves of respiration and blood pressure and convulsive movements were especially noted.

It was found that a number of local anesthetics which were examined along with other drugs were readily absorbed through the praeputium. Figure 6 shows this phenomenon in a striking manner. After cocain, fluctuations in the respiration and blood pressure, as well as convulsive movements of the muscles, were noted. After apothesin, very rapid poisoning of the animal occurred, following the introduction of two tablets of the drug into the praeputial sac.

ABSORPTION THROUGH THE VAGINA

An experimental study of the subject of absorption of drugs and poisons through the vagina, as well as a review of the clinical toxicological literature of the same, was published in this journal by the author [3] some time ago. In the present communication, reference need be made only to the absorption of local anesthetics by that route.

That local anesthetics are readily absorbed through the vaginal wall is readily illustrated, in Figures 7 and 8. In Figure 7, a 4 per cent. solution of cocain hydrochlorid, 5 c.c. was introduced into the vagina of a dog. It will be noted that after the lapse of a few minutes, constitutional symptoms of poisoning developed. The respirations were first stimulated and then depressed (not shown in the figure); the animal developed convulsions; and the blood pressure curve shows first a drop and later a rise, due to the convulsive contractions of the muscles.

Figure 8 illustrates the effect of introducing two tablets of apothesin into the vagina of a dog. It is interesting to compare this figure with Figure 9, in which 4 c.c. of a 1 per cent. solution of apothesin were injected intravenously in another dog. It will be seen that the poisonous effect of the drug is almost as marked in the first case as in the second.

DISCUSSION

As a result of the various experiments recorded above, it is evident that local anesthetics are very easily absorbed through various organs of the genito-urinary tract, with the exception of the bladder. Of great scientific as well as practical interest is also the difference between the absorptive powers of the urethra as compared with the bladder. Whereas, drugs are very easily taken up through the urethral walls, they are very slowly absorbed, indeed, from the bladder. These experimental data agree very well with the experiences of both urologists and gynecologists, so far as the author has been able to ascertain. The

3. Macht: Jour. Pharmacol. & Exp. Therap. **10**:509, 1918.

absorption of cocain through the ureter and pelvis of the kidney is chiefly of scientific interest; and yet, in view of the modern use of drugs, such as iodides, thorium, etc., in these structures, the subject may also be of some practical importance. Absorption through the prepuce is important from the surgical point of view, as local anesthetics are very widely employed in this connection, and their careless or excessive use may lead, and has led, to toxic symptoms in patients. The absorption of local anesthetics from the vagina is also interesting, even though these drugs are not so widely introduced into that canal as other poisons are, such, for instance, as the antiseptics, mercuric chloride, phenol, lysol, etc.

SUMMARY

1. The local anesthetics, cocain, alypin and apothesin, were studied in regard to their penetration through an absorption from various genito-urinary organs.

2. It was found that these drugs are more or less readily absorbed through the urethra, ureters, pelvis of the kidney, praeputium and vagina.

3. It was found that while the local anesthetics are readily and rapidly absorbed from the urethra, they are very poorly absorbed from the urinary bladder.

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TINCTURE OF DIGITALIS AND THE INFUSION IN THERAPEUTICS*

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AND
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Our knowledge of the pharmacology of the digitalis group is increasing rapidly, and its therapeutic use now begins to approach an exact science more nearly, perhaps, than that of all excepting a few drugs. But there are still many minor points, and not a few major ones, that require further study.

Clinicians have long held the opinion that the action of the tincture of digitalis differs qualitatively as well as quantitatively from that of the infusion. This difference is usually explained on the ground that the several active principles found in the leaf are not extracted in the same relative amounts by the menstruum used in making the tincture and the water used in making the infusion.

Pharmacologists have shown a disposition to give too little consideration to the views of the older therapeutists when these appear to be in conflict with the deductions drawn from the results of modern experiments, even as many therapeutists have a tendency to dismiss pharmacologic deductions when they fail to support opinions based on clinical observations. Obviously, we can accomplish no more through the clinical use of a drug, however extensive our knowledge of its pharmacology may be, than was accomplished formerly by one who chanced to discover the correct way of employing it, and it is probable that William Withering used digitalis with success equal to that of many of the clinicians of today.

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* From the Laboratory of Pharmacology of Cornell University Medical College.

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

Nothing could be further from our purpose than to attempt to belittle exact pharmacologic investigations; but it is true that pharmacologists and chemists are responsible for much of the misunderstanding that exists today regarding this drug. On the other hand, many of the older clinicians were acute observers; and, while their deductions may have been faulty at times, these were often founded on fact. This should serve as a warning to us against dismissing long established clinical opinions without attempting to understand the basis for their existence, and to explain wherein the error lay when such is proved to exist. With the clearing away of misconceptions, the use of the drug will approach the state in which the general practitioner will be able to use it even more wisely than Withering employed it. We shall therefore discuss briefly the basis for the view that the constituents of the infusion of digitalis differ in kind from those of the tincture.

It is not our purpose to discuss the chemical nature of the active principles of digitalis, or their solubilities except as may be necessary for an understanding of the question of the composition of the tincture and the infusion.

COMPOSITION OF TINCTURE OF DIGITALIS

Digitoxin, digitalin and digitalein, the three principles generally supposed to represent the activities of the leaf, are soluble in alcohol, and all of these (if, indeed, they exist in the leaf) are present in the tincture; and it may be stated at once that the official tincture represents the activity of the leaf completely, that is to say, that the tincture contains all except traces of the active principles of the dried and powdered leaf from which it is made.

There is no way of estimating the total amounts of the several active principles in the leaf or in the tincture directly; therefore the following method was used in order to determine whether the tincture represents the leaf fully:

A tincture was prepared according to the directions of the United States Pharmacopeia, and the marc (the drug left in the percolator after extraction) was dried and weighed. The dried marc weighed just half as much as the drug weighed previous to extraction; this was odorless and tasteless, or nearly so. This itself points strongly to the removal of all the active principles during percolation, for all of them

are very bitter, and nothing is destroyed during the simple process of extraction with the menstruum consisting of three volumes of alcohol and one of water.

An infusion was then prepared by pouring 1,000 c.c. of boiling water on 7.5 gm. of the dried marc, representing 15 gm. of the original digitalis from which the tincture had been made, and this infusion was tested in order to determine whether it contained any active substance.

The intravenous injection of 150 c.c. of this infusion of the marc per kilogram of body weight was without perceptible effect, though this dose was equal in volume to about twenty-five times that required of an infusion of this specimen of digitalis to cause death. The cat then received a further intravenous injection of the full average fatal dose of ouabain before death resulted, showing conclusively that the infusion of the marc (and the marc itself) contained not more than traces of water-soluble active digitalis principles.[1]

The presence of saponin-like bodies (digitonin or digit-saponin) in digitalis has given rise to much discussion. These substances are devoid of the therapeutic action of digitalis, but on the other hand, they may be deleterious when large amounts are injected intravenously, and their significance in digitalis therapy is frequently misunderstood. The gastric disturbance which sometimes follows the oral administration of tincture of digitalis has been attributed largely, but erroneously, to these substances, though Cushny [2] states that the tincture contains less of these than the infusion. Presumably Cushny means the tincture made from a given weight of drug contains less than the infusion, which represents an equal weight of the drug.

We are not aware of any direct experimental evidence on which this statement is based, but one frequently sees it coupled with the explanation that these saponin bodies are insoluble in alcohol. This deduction is misleading in that while they are insoluble in absolute alcohol, the official tincture is made, as previously stated, with a menstruum of about 70 per cent. alcohol. We have therefore attempted to determine whether the

1. The use of ouabain in this experiment is based on what is termed the "combined ouabain" method for the estimation of small amounts of digitalis bodies, which was described by Hatcher and Brody (Am. J. Pharm. **82**: 360, 1910). This method consists in determining the difference between the average fatal vein dose of ouabain for the cat per kilogram of weight and that required to kill after the previous injection of a dose of any digitalis body. The difference represents the percentage of the fatal dose that is attributable to the digitalis body under investigation.

2. Cushny: Pharmacology and Therapeutics or the Actions of Drugs, 1918, p. 399.

marc left after preparing the official tincture contains saponin-like bodies or not, and if so, the approximate amount as compared with that present in the official infusion, though the matter did not appear to us to be of great practical importance, because it is well known to pharmacologists that the leaf contains only small amounts of these bodies, and even these amounts are not absorbed readily from the gastro-intestinal tract, nor do they cause local irritation in amounts at all comparable to those that can ever be administered during the therapeutic use of digitalis.

PRESENCE OF DIGITONIN

Kiliani,[3] who is probably the greatest living authority on the chemistry of digitalis, states that digitalis leaves contain only traces of digitonin, and he also says [4] that Boehm found that digitonin causes gastric irritation in dogs only after the oral administration of enormous doses. These results attributed to Boehm have been confirmed by experiments in this laboratory. Unfortunately for the cause of scientific therapy, the fallacy regarding the supposed rôle of digitonin in the production of gastric disturbance following the administration of the tincture of digitalis in therapeutic doses appears to us to have been kept alive largely from motives of financial interest; at least, we have been unable to discover any evidence to support the theory mentioned, and an advantage sometimes claimed for proprietary substitutes for the official preparations of digitalis is that they lack the irritant or disturbing effect of these saponin-like bodies.

We undertook to compare the laking action of an infusion prepared from the powdered leaf with that of one made from the marc of the tincture, but the laking action exerted on partially washed corpuscles (obtained by mixing blood with 100 parts of physiologic sodium chlorid solution and allowing the corpuscles to settle to the bottom of the vessel) was so feeble in both cases that we abandoned further effort in this direction.

We found that the intravenous injection of digitonin (Merck's) in doses of 20 mg. per kilogram of body weight caused hematuria in three experiments on cats, and, on the other hand, the intravenous injection of an

3. Kiliani: Arch. d. Pharm. **243**: 7, 1905.
4. Kiliani: Arch. d. Pharm. **230**: 260, 1892.

infusion of the marc of the tincture in doses of 150 c.c. per kilogram of weight produced no perceptible effect. From this we may conclude that very little digitonin remains in the marc of the tincture and whether or not the tincture contains more than traces of it, the difference between the amounts of it present in the tincture and in the infusion of corresponding amounts of the drug are of no therapeutic or toxicologic importance.

The subject does not seem to us worth further consideration in view of what has been said, especially since we have such high authority as Kiliani for stating that the leaf contains only traces of digitonin.

The Pharmacopeia gives directions for the biologic assay of digitalis with the standard of activity required for the leaf, the fluidextract and the tincture, but none for the infusion; it is far more important to the clinician, therefore, to know to what extent the infusion represents the leaf from which it is made than to know whether the tincture contains more than traces of saponin bodies.

DIGITALEIN AND DIGITALIN

Digitalein is very soluble in water, and it may be accepted that any that may be present in the leaf passes into the infusion. Digitalin (the true digitalin of Schmiedeberg and of Kiliani) is also extracted by water, possibly with the aid of the saponin bodies; but neither digitalein nor digitalin is absorbed readily from the gastro-intestinal tract of animals or that of man.

Oral doses of digitalein equal to the fatal vein dose were administered to cats daily for periods of a week, and it was then found by means of the combined ouabain test that there was little persistence of action. Amounts of digitalein equal to four or five times the single fatal vein dose were administered to each of several cats through a stomach tube in single doses without inducing perceptible effects.[5]

Schmiedeberg[6] states that the absorption and behavior of digitalein are such that it is difficult to

5. Unpublished experiments performed in this laboratory some years ago and recently confirmed by us in part. The digitalein of commerce is said to consist of a mixture of glucosids from Digitalis purpurea, prepared according to the process of Schmiedeberg, and containing digitoxin, digitalin and digitalein (New and Nonofficial Remedies, 1920, p. 88); and since the proportions of these three appear to vary in different specimens of commercial digitalein, we do not pretend to say that all specimens show the same behavior. In fact, it is our opinion that digitalein has no place in the materia medica while it remains so uncertain in composition and activity.

6. Schmiedeberg: Arch. f. Path. u. Pharm. **62**: 305, 1910.

obtain therapeutic effects with it, and that digitalin is absorbed slowly and irregularly from the gastro-intestinal tract. Naunyn [7] states that German digitalin (which consists of true digitalin in part) exerts only a weak and uncertain action after its oral administration to man; and Eggleston [8] found that both digitalein and digitalin are absorbed so poorly from the gastro-intestinal tract of man (as judged by the lack of therapeutic effects) that they are unsuitable for therapeutic use.

ABSORPTION OF DIGITOXIN

Digitoxin is practically insoluble in water, and it is commonly stated that the infusion contains only traces of it,[2] or that it contains more or less of the digitoxin held in suspension by the saponin bodies. Sollmann [9] states that a 1 : 10 infusion (note that this is not the official) contains two thirds of the digitoxin of the leaf.

The evidence that is available to us appears to show incontrovertibly that digitoxin is absorbed from the gastro-intestinal tract of man with a far greater approach to uniformity than is either digitalin or digitalein. It is only proper to state that Schmiedeberg [6] states that the insoluble digitoxin is absorbed even less regularly than digitalin, the administration of a given amount causing pronounced effects in one case and none in another. This is apparently an example of erroneous deduction, following the view so commonly held that insolubility in water exerts a profound influence on the absorption of even those drugs which are given therapeutically in doses of a milligram. Solubility does play an important rôle in the absorption of drugs which are given in doses of a gram or more, but this factor is of minor importance with reference to the absorption of such minute amounts as one gives of digitoxin.

Naunyn [7] used digitoxin, and states as the result of clinical experience that it is absorbed completely, despite its insolubility (in water). Huchard [10] comments on the frequent lack of therapeutic success with different specimens of digitalis and says that he always gives the preference to crystalline digitaline (digitoxin)

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7. Naunyn: Therap. d. Gegenw. **1**: 193, 1899.
8. Eggleston, Cary: Digitalis Dosage, Arch. Int. Med. **16**: 1 (July) 1915.
9. Sollmann, Torald: Manual of Pharmacology, Philadelphia, W. B. Saunders Company, 1917, p. 383.
10. Huchard: Les maladies du cœur, 1908, p. 164.

because it is uniform in its therapeutic effects. Eggleston [8] also found the absorption of digitoxin from the gastro-intestinal tract of man to be more nearly uniform than that of digitalin or digitalein; at least, he found the dose required to be less variable.

AMOUNT OF DIGITOXIN IN THE INFUSION

Having submitted evidence that appears convincing to us that digitoxin is absorbed from the gastro-intestinal tract of man, with a far greater approach to uniformity of action than is the case with digitalein or digitalin, it is increasingly important to determine whether the infusion contains only traces of digitoxin, as Cushny [2] states, or much larger amounts, as Sollmann [9] claims, for it is evident from the foregoing that the therapeutic value of the infusion must depend on the presence in it of digitoxin, if there are only these three active principles in the leaf in important amounts, or on the presence of some other readily absorbable principle not described heretofore.

There is no way of separately estimating the several active principles of the infusion, and we have approached the solution of the question in the same way that we determined that the tincture represents the leaf completely, that is, we examined the marc left after the infusion had been made.

VARIOUS METHODS OF MAKING INFUSION OF DIGITALIS

Before presenting the results of these experiments, we wish to discuss the several methods that we have employed in making the infusion, including that official in the United States Pharmacopeia, and a modification that we have used in this laboratory for several years for the preparation of infusions for use in experimental studies.

United States.—The United States Pharmacopeia directs that the infusion shall be prepared by pouring 500 c.c. of boiling water on 15 gm. of bruised digitalis and allowing it to macerate for one hour, after which the liquid is strained, 500 c.c. of cinnamon water is added to the strained liquid, and enough water is passed through the strainer (and the marc) to make 1,000 c.c.

It would be interesting to know why the Pharmacopeia directs that the drug be "bruised" instead of directing that

the powdered digitalis be used. According to the pharmacopeial requirements, the leaf must be "carefully" dried, but there is no statement as to the amount of moisture allowed. The lamina of digitalis is brittle, when properly dried, and one does not speak of "bruising" a brittle substance; it is only the tough and useless midribs that resist trituration, and their presence serves to prevent the reduction of the lamina to a uniform powder, unless the trituration is prolonged; hence the degree of fineness of the "bruised" digitalis will vary widely, depending on the interpretation put on the direction to bruise the drug, and also on the amount of the contained moisture and the relative amount of midribs.

It will be observed also that only 500 c.c. of water is used in the extraction of the drug in making 1,000 c.c. of the official infusion, since the cinnamon water, constituting half of the total volume, is added to the strained liquid and not passed through the marc on the strainer. Furthermore, not all of the infusion held in the marc is removed by the water which is passed through the strainer to make up the required volume.

British.—The British pharmacopeia directs that the infusion be prepared by pouring 1,000 c.c. of boiling water on 7 gm. of digitalis in No. 20 powder, allowing it to stand for fifteen minutes, and straining while hot. It will be observed that this process involves the use of less than half as much drug as that of the United States Pharmacopeia: that a uniform powder is directed; that the solvent action of all of the liquid is utilized, though for a shorter time, and that the finished infusion is not diluted by passing water through the strainer.

Author's Method.—The infusion that has been used so frequently in this laboratory for experimental purposes has been prepared essentially as follows: One thousand c.c. of boiling water is poured onto 10 gm. of digitalis in No. 60 powder in a flask or beaker, which is allowed to stand for one hour in a boiling water bath with frequent stirring of the infusion, in order to expel the air from the cells, thus facilitating extraction, water being added to replace that lost by evaporation; the infusion is cooled, and filtered through paper, or filtered while hot, when it is desired to maintain it in a sterile condition.

PRACTICAL TESTS OF VARYING METHODS

Infusions were prepared in the official way and in that just described, after which the marc was washed free of the adhering infusion, or this was gotten rid of by pressing the marc between layers of filter paper, adding a small volume of water and again expressing. The marc was then dried and weighed, after which tinctures were prepared and examined for their activity

by testing them on cats. We are aware that the washing of the marc with about 200 c.c. of water involves a slight error; but if 1,000 c.c. of boiling water fails to extract the active principles, we do not believe that the error involved in washing this marc with 200 c.c. of cold water is of any importance. In some instances we estimated the activity of the infusions directly, in order to compare the activity of those prepared in different ways.

RESULTS

The results of these experiments may be presented briefly without giving the protocols in detail.

An infusion made according to the official (U. S. P.) formula was not quite so active as one made in the same way, except that 1,000 c.c. of boiling water was poured on the "bruised" leaf. The activity of the official infusion was such that 6.3 c.c. was required for each kilogram of body weight of the cat to cause death (average of two fairly concordant experiments), while an average of 4.4 c.c. was required of the second infusion per kilogram of weight (average of two fairly concordant experiments). It will be seen that the activity of the official infusion was only about 70 per cent. of that of the second, which represented about 90 per cent. of the activity of the leaf; at least, the tincture prepared from the marc of the second specimen was about one-tenth as active as an average tincture, while the tincture prepared from the dried marc of the official infusion was about one-fifth as active as the average tincture of good quality. These tinctures were evaporated to expel the alcohol, after which they were diluted with physiologic sodium chlorid solution before being tested on cats in the usual manner.

When the infusion was prepared by pouring 1,000 c.c. of boiling water on 15 gm. of digitalis in No. 60 powder, its activity, indicated by the direct tests on cats, was equal to that of the tincture prepared from the same amount of this specimen of drug, and, since we have seen that the tincture exhausted the drug completely, it would appear that the infusion also represented the full activities of the powder in this case. However, the activity of the tincture made from this marc was equal to about 4 per cent. of the activities of the leaf, the discrepancy being within the limits of error, so far as the direct test of the infusion (two experiments) is concerned; but the test of the tincture of the marc showed conclusively that at least a very small part of the active principles actually remained in the marc.

The infusion made by pouring 1,000 c.c. of boiling water on 10 gm. of the No. 60 powder represented the drug even more completely than in the case just mentioned, and the tincture

prepared from the dried marc of this infusion was practically inert; at least, the injection of the tincture (after evaporation of the alcohol) representing the marc of 3 gm. of the drug was without effect, and the animal then required 90 per cent. of the average fatal dose of ouabain to cause death, indicating that the marc was about one three-hundredth part as active as the drug before infusion. This affords satisfactory evidence that 100 parts of boiling water suffice for the extraction of all of the active principles of the drug in No. 60 powder when maceration is continued for an hour.

In one case the drug was used in the form of No. 80 powder in the proportion directed by the Pharmacopeia, 15 gm. for 1,000 c.c. of infusion, after which the activity of the marc was equal to about 3 per cent. of that of the original powder. This indicates that the finer powder is somewhat more nearly exhausted by that volume of water than is the coarser, since the activity of the marc of 15 gm. of No. 60 powder was slightly greater than this;[11] but when a smaller proportion of drug is used, the finer powder is not necessary, and an infusion made from the latter is tedious to strain or filter.

VARIABILITY OF OFFICIAL INFUSION

It hardly required the present study to arrive at the conclusion that the official infusion lacks uniformity. The long standing and extreme diversity of competent clinical opinion alone would suggest this strongly, and the method of completing the required volume of the finished product by passing water through the strainer must result in some variation in the activity of the infusion, even though the formula were faultless in other particulars. A given volume of infusion is made from 15 per cent. of the weight of the drug that is used in making such a volume of the tincture, but the activity of the average infusion is probably equal to less than 10 per cent. of that of the tincture, as indicated by the biologic test, and the difference in the therapeutic effectiveness is probably much greater as a rule, because the deficiency in extraction in making the infusion falls wholly on the least soluble but the most absorbable fraction, the digitoxin or the digitoxin-like substance.

From this a somewhat paradoxical situation arises. It is well known that many druggists prepare a so-called infusion by diluting the tincture or fluid-

11. We have found that the removal of air from the cells of the drug by means of a vacuum pump facilitates extraction with water. The particles of a No. 60 powder have a diameter of 0.23 mm., and those of a No. 80 powder, 0.17 mm.; hence the latter are less than half as large as the former.

extract, while those who take pride in their profession make the infusion according to pharmacopeial directions, and it may happen frequently that the so-called infusion prepared from the fluidextract will contain more of the digitoxin than a strictly official infusion prepared with great care, and the preparation that should be condemned by all rules of scientific pharmacy will then give better therapeutic results than the one prepared conscientiously. This is in no sense an argument for slipshod pharmacy, but it is an argument for improvement in the official formula for preparing the infusion.

ADVANTAGES OF INFUSION PREPARED BY METHOD HEREIN DESCRIBED

The infusion prepared in the manner described, in which 1 part of digitalis in No. 60 powder is treated with 100 parts of boiling water and kept for one hour in a boiling water bath with frequent stirring, contains all of the active principles of the leaf. When this infusion is filtered and used therapeutically, the effects are the same as would be induced by the tincture in doses just one tenth as large. If one desires the cinnamon flavor, this may be secured by adding 1 c.c. of oil of cinnamon to 10 gm. of the powdered digitalis previous to the addition of the boiling water. Very little of the volatile oil is lost during the process of preparing the infusion.

An infusion thus prepared has these advantages over one made according to the present Pharmacopeial method:

1. There is better extraction, whereby the infusion represents the activities of the drug completely.
2. There is uniformity of activity, in place of the variability of the official.
3. With a given degree of activity it contains a larger proportion of the slightly soluble, but more readily absorbable, digitoxin or digitoxin-like substance, or substances.
4. The dosage is just ten times that of the tincture in volume.
5. The filtered infusion is transparent.
6. It may be kept indefinitely without loss of activity.

STABILITY OF INFUSION

We are not at present concerned primarily with the question of the stability of the infusion, but we wish to

correct some erroneous views that are prevalent concerning the question, for it is useless to show the way to make an infusion properly if the preparation is valueless, because of the rapidity with which it decomposes, as some believe.

No one denies that the infusion of digitalis decomposes under adverse conditions—like all other infusions of drugs that contain no antiseptic—but the question that concerns the clinician who, for any reason, prefers to employ the infusion, is whether it can be prepared conveniently in a reasonably stable form. Hatcher and Eggleston [12] have shown that the infusion of digitalis prepared in the manner recommended here, and filtered while hot, retains its activity with little change for several weeks when kept with reasonable care. We are able to offer evidence confirmatory of this conclusion.

One of us (R. A. H.) prepared an infusion of digitalis in the manner described, using 1 part of the powdered leaf to 100 parts of boiling water, filtering the infusion while hot, putting it into bottles which it filled completely, corking these, and sealing them with paraffin, Feb. 5, 1918. Specimens of this infusion have been tested on cats from time to time, and no perceptible loss of activity could be detected at the time of the last examination. A slight precipitate has formed in every bottle, leaving the supernatant fluid perfectly transparent, and in a few there has been a growth of mold, owing to imperfect sterilization of the cork.

THERAPEUTIC EFFECTIVENESS

Dr. Cary Eggleston used a specimen of this infusion clinically, July 28-29, 1920, and found the dose required to induce the typical therapeutic effects to be the equivalent of that which he [8] had established for the tincture as measured in terms of cat units. A condensed report of the essential facts is presented here through the courtesy of Dr. Eggleston. The apex rate was 145, and the radial pulse rate was 73, the pulse deficit being 72 after two days' rest in bed; the infusion was then administered at 12 midnight, 6 a. m. and at 12 noon. The apex rate was reduced to 104; the radial pulse rate rose to 86, the deficit being 18, shortly before the last

12. Hatcher, R. A., and Eggleston, Cary: The Stability of the Infusion of Digitalis, J. A. M. A. **65**: 1902 (Nov. 27) 1915.

dose. The condition steadily improved and the pulse deficit had nearly disappeared within four hours after the last dose, and on the following morning this deficit had disappeared completely, the rate being 66.

The condition of the heart, as shown by the pulse deficit, was worse at the time the first dose of the infusion was administered than it had been twenty-four hours before, showing that rest in bed alone was not producing any improvement. The striking improvement that occurred within less than twelve hours hardly leaves room for doubt concerning the therapeutic effectiveness of this specimen of infusion that had been prepared nearly two and a half years previously. The infusion was filtered before being used, showing that the precipitate had not carried down the active principles in an insoluble form.

SUMMARY

1. Tincture of digitalis was prepared, the marc of which was dried and used in the preparation of an infusion; this infusion of marc was tested on cats and found to be inert, showing that all of the active water-soluble principles of the leaf are extracted during the percolation for making the tincture.

2. This method of testing the marc affords a delicate means of testing the degree to which the active water-soluble principles are extracted during the percolation of the drug.

3. There is no essential difference in the amounts of the saponin bodies present in the tincture and in the infusion prepared from equal weights of the leaf, and therapeutic doses of digitalis do not contain enough to induce any undesired effects.

4. Infusions of digitalis were prepared in different ways. In each case the marc was washed and dried, after which it was used for the preparation of tincture, and this tincture was tested on cats in order to determine to what extent the active principles had been extracted during the preparation of the infusion.

5. The official infusion does not represent the drug completely; hence the standardization of the leaf does not insure uniformity in activity of the infusion. The variability of the infusion is at the expense of the more absorbable of the active principles.

6. The infusion prepared according to the simple method described represents the activities of the leaf completely; hence it permits of uniformity when a standardized powder is used for making it. It may be used in place of the tincture in doses just ten times the volume of those of the latter, and it becomes a matter of indifference, so far as therapeutic effects are concerned, which is used.

7. We have been unable to discover any experimental evidence to support the view, still held by many, that there is a necessary qualitative difference between the actions of the tincture and those of the infusion of digitalis, even when the latter is prepared properly.

8. An infusion of digitalis prepared in the manner recommended, and kept in completely filled and hermetically sealed bottles for more than two years and five months, retained its activity unimpaired, as shown by the results of tests on cats and by the therapeutic effects on man.

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ASTRINGENCY AND PROTEIN-PRECIPITATION BY MASKED TANNIN COMPOUNDS*

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INTRODUCTION

The use of tannins in diarrhea has led to the introduction of a number of compounds that are intended to avoid any action in the stomach, and to develop astringent effects in the intestine. In principle, these are based on the differences in reaction (hydrogen-ion concentration) that prevail at the different levels in the alimentary tract; i.e., the preparations are intended to be insoluble in the acid stomach, but to dissolve and become astringent in the intestines.

These properties are claimed, at least by inference, for numerous commercial tannin compounds.

With the hope of arriving at an independent and objective classification of these agents, it appeared worth while to check up the data as to solubility and as to astringency, under uniform conditions that would simulate those of the digestive tract, at least as to the degree of reaction (hydrogen-ion concentration) and time of contact.

The solubility determinations were undertaken by Dr. P. N. Leech. His results are being published in detail elsewhere; but they have such important relations to astringency, that brief abstracts of them will be given in this paper.

However, the solubility of a tannin-compound in itself does not guarantee astringency. It is necessary that the solution contain the tannin free, or in such a form that it may react with protein. This can only be known by the direct experiments which form the main portion of this investigation.

There are several methods of demonstrating astringency, by the precipitation of proteins or on excised tissues, or on living animals. As the relative value of these was not known, several methods were tried, including a new modification, viz., antihemolysis by tanning red corpuscles. These, and the results obtained with them, will be described seriatim.

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* From the Department of Pharmacology of the Medical School, Western Reserve University.

Method of Determining the Solubility.—Leech investigated the solubility under conditions approximating those of the body; i.e., by digestion at 40 C. for three and one half hours, with frequent agitation. The undissolved residue was then collected on a filter, dried, and weighed. One gram of the tannin-preparation was used for 250 c.c. of solvent. The solvents were:

Water.

Dilute acid: N/12.5 (0.2 per cent.) HCl.

Acid-pepsin: This acid, with 0.1 per cent. of pepsin.

Alkaline: Sodium bicarbonate 1 and 2 per cent. These gave practically identical results.

Alkaline trypsin: Bicarbonate with 0.05 per cent. of holadin.

Results of Solubility Determinations. These are summarized in Table 1.

TABLE 1.—SOLUBILITY (RANGE AND MEDIAN) OF MASKED TANNIN COMPOUNDS

Type	Water	HCl	HCl with Pepsin	Bicarbonate	Bicarbonate with Holadin
Tannalbin	18-22 (21)	32-34 (33)	56-87 (75)	41-52 (42)	45-68 (58)
Albutannin	15-16 (16)		95-98 (96)	31-34 (33)	77-86 (82)
Protan	14-16 (16)	30-38 (35)	73-81 (77)	90	81-98 (94)
Acetannin and tannigen	6.1-17 (8.5)	(6.2)	3.8-17.4 (5.8)	31-100 (82)	32-100 (76)
Tannoform	21-47 (32)	32-42 (37)	12-21 (15)	81-95 (87)	78-96 (84)
Tannopin	17	32	20	26	24
Tannismuth	18	25	15	15	13
Gallogen	18	6.2	16	15	17

All the commercial masked tannin compounds depart more or less in solubility from the claims made for them; and there are quite wide variations in different specimens of the same product.

Solubility in water: None are insoluble, or even "practically insoluble" in water. This, however, has probably but little therapeutic importance.

Solubility in dilute acid: Acetannin comes nearest to being insoluble in the acidity of the gastric juice. All the other compounds dissolve fairly readily.

The presence of pepsin increases the acid solubility of tannin albuminate materially, presumably by dissolving the heat-coagulated albumin. With the other compounds, pepsin either has no effect or the solubility *appears* decreased through the tannin precipitation of the protein of the pepsin.

Insolubility in the gastric juice would be desirable, theoretically, by precluding undesired gastric effects. In fact, however, it is probably not important, provided that the solution is slow, or if the tannin is taken with food.

The solubility in bicarbonate varies quite widely. With the albumin-tannates, it is materially aided by trypsin. The speed of solution, and the liberation of tannin from its esters, are also factors of practical therapeutic importance.

Rapid solution and liberation of the tannin would be favorable to intensive but short action in the upper intestine; whereas slow solution or liberation would be necessary for prolonging the action into the lower intestines.

From this standpoint, the compounds would therefore fall into the following groups:

(*a*) Compounds easily soluble in reactive form, and therefore likely to exhaust their action in the upper intestine: protan.

(*b*) Compounds with protracted action because slowly soluble: Albutannin.

(*c*) Compounds with protracted action because slowly hydrolyxed: Acetannin.

(*d*) Compounds that are less than half soluble. They are probably mixtures, and it would be difficult to predict their actions from their solubility: Tannoform, tannopin, tannismuth, gallogen.

THE PRECIPITANT ACTION OF MASKED TANNIN COMPOUNDS ON EGG ALBUMIN

It has been shown that these compounds are, for the most part soluble in acid, and usually somewhat more soluble in alkaline media. The next question was whether these solutions were able to precipitate proteins at reactions corresponding to the acidity of the stomach and the alkalinity of the intestines. One method of approaching this was by estimating the diminution of protein nitrogen when they were left in contact with an egg albumin solution. This constitutes a good method for comparing the products. The experiments were carried out by O. H. Schettler and N. C. Wetzel.

Method. The albumin was used in 5 per cent. solution of the moist egg white in N7/100 HCl and in 1 per cent. sodium bicarbonate, respectively. To 50 c.c. of these, there was added 1 gm. of the tannin drug. The mixture was set aside, with frequent shaking, for twenty-four hours, centrifuged, and the nitrogen determined by the Kjeldahl method in duplicate samples of 5 c.c. of the clear fluids. In the albumin solutions, without tannins, the nitrogen amounted to 6.2 to 6.8 mgm.

In the case of the various brands of tannalbin, the drug was also macerated without the egg albumin, and the nitrogen of its solution determined.

Each series was carried through as a single operation.

Results with Egg Albumin Precipitation. These are compared in Tables 2 and 3. They show that:

The tannin albuminates alone precipitate albumin in acid solution. This indicates that they have least perfectly attained the therapeutic object of avoiding precipitation in the stomach.

All the classes precipitate albumin in alkaline solution, the individual samples varying quite as much as the classes.

The tannigen class represents most perfectly the theoretical therapeutic objects of nonprecipitation in acid reaction (stomach) and extensive precipitation in alkaline reaction (intestines).

Tannoform, tannopin and gallogen, however, also fulfill these objects.

The protein of commercial tannin albuminates is somewhat soluble in both acid and alkaline media, and to about the same degree.

TABLE 2.—PERCENTAGE OF ALBUMIN PRECIPITATED BY TANNINS

	HCl n 7/100	Sodium Bicarbonate 1 Per Cent.
Tannin	63	100
Tannigen (1)*	0	62
Acetannal	0	96
Tannalbin (2)*	26	76
Tannin Alb Exsic (24)*	26	39(?)
Albutannin (Protannal)	35	97
Tannoform (4)*	0	57
Tannopin (12)*	0	50
Gallogen (11)*	0	58

* These are the code numbers of the specimens. A detailed list of these is given in Leech's paper.

TABLE 3.—SOLUBILITY OF THE PROTEINS OF COMMERCIAL TANNIN ALBUMINATE, IN TERMS OF MILLIGRAMS OF NITROGEN DISSOLVED PER HUNDRED MILLIGRAMS OF DRUG

	HCl n 7/100	Sodium Bicarbonate 1 Per Cent.
Tannalbin (2)	2.0	1.6
Tannin Alb Exsic (24)	2.0	0(?)
Protannal	2.3	3.8

SERUM PRECIPITATION AS A CRITERION FOR ASTRINGENT ACTION OF MASKED TANNINS

Inasmuch as astringency is probably proportional to protein precipitation, any convenient protein should be suitable for comparative tests. In this series, a sample of dried serum was used, as it permitted the making of solutions of uniform concentrations at different times. The experiments were planned to illustrate the influence of various reactions, concentrations, etc.; and were generally combined with hemolytic and other tests, to determine the practical value of the different methods. The relations will be brought out later.

General Course of Experiments.—The typical experiments consisted in placing into numbered test tubes 10 c.c. of the *solvents* (see below); then the weighed quantity of the astringent drug. The tubes were then stoppered, shaken and digested at 40 C. for four hours, inverting the tubes every hour. At the end of this time, these *tannin digests* were filtered. The taste of the solution

was noted for astringency. Then 5 c.c. of the filtrate were made neutral to methyl orange by HCl and bicarbonate (i.e., pH = 3.1 to 4.4), and added to 5 c.c. of the *serum solution* (see below).

The occurrence of precipitation was observed, at once, and again after one to three days. Observations were also made on the solubility of some of the precipitates in sodium bicarbonate.

The following solvents were used:

I. "NaCl," 0.9 per cent. (i. e., isotonic saline).

II. "HCl," 7 c.c. of normal in 100 c.c. of 0.9 per cent. NaCl. This corresponds to about 0.25 per cent. of HCl, and to pH = about 1.16. It was intended to represent the acidity of the gastric juice.

III. "Pepsin," 0.25 per cent. of pepsin in "HCl" II; intended to represent gastric juice.

IV. "Bicarb," Sodium bicarbonate 0.5 per cent. in NaCl, 0.9 per cent.; representing the alkalinity of the intestine.

V. "Trypsin," 0.25 per cent. of trypsin in "Bicarb" IV; representing pancreatic juice.

VI. "Bicarb-bile," bile salts (bilein) 0.063 per cent. in "Bicarb" IV; representing the bile.

VII. "Carb," sodium carbonate, 1 per cent.

Serum solution. This was made by rubbing 2.5 gm. of dried and powdered serum in a mortar with 0.9 per cent. NaCl to a total of 250 c.c. It was left to stand half an hour, and filtered through filter paper.

Results. These will be discussed later, under the individual drugs.

BLOOD-CORPUSCLE METHOD OF TESTING ASTRINGENCY[1]

The precipitation of proteins in cell membranes is assumed to render these less permeable, analogous to tanning. Accordingly it was to be expected that treatment of red blood corpuscles with astringent solutions would render them resistant to the laking action of water; i.e., that astringency would result in antihemolysis. This supposition was confirmed by experiment. When a 1:10 dilution of defibrinated dog's or sheep's blood in 0.9 per cent. NaCl is added to a 1 per cent. tannin (1:10) solution in 0.9 per cent. NaCl, a fawn colored precipitate of corpuscles is formed. This is allowed to stand for five minutes. Water may then be added (10:1) without the occurrence of laking, even after four days.

Threshold Concentration of Tannin.—Twenty-five hundredth per cent. was found perfectly effective; 0.0625 per cent. was doubtful.

Laking by Solvents.—The solvents used in the digestion experiments were tested on the corpuscles by a similar technic. HCl N / 15 produced direct laking when added to the blood suspension; but not, of course, if the acid was first

1. About eighteen months after these experiments were completed, I find that Kobert (1918) has also published a "blood-corpuscle method" for the quantitative evaluation of astringency. His method, however, is quite different from that used by me, for it measures the agglutinating power, and not the protection against hemolysis.

neutralized. Acid digests were therefore neutralized before testing. Sodium bicarbonate, 0.5 per cent., does not produce laking. Bile salts would produce laking and therefore were not used in making digests.

Antihemolysis by Masked Tannin Compounds in Various Media.—This was investigated in several series of experiments. Since they differ only in minor details, they may be discussed together.

General Method.—The subsequent experiments were carried out according to the following method:

Tannin Digests.—These were prepared as explained under serum precipitation. In fact, the same digests were used in some of the experiments, thus permitting the direct comparison of the two methods.

All solutions contained 0.9 per cent. NaCl.

Blood Suspension.—Ten per cent. of defibrinated dog's or sheep's blood in 0.9 per cent. NaCl.

Mixture.—The digests were filtered and used either directly or after neutralization. To 5 c.c. of the digest was added 0.5 c.c. of the blood suspension; the mixture set aside for half an hour, and *direct laking* observed.

Two cubic centimeters of the mixture was then added to 20 c.c. of water, set aside, and observed for *water* laking.

Results.—These will be discussed under the individual drugs.

Astringent Taste of Solutions.—Nothwithstanding its subjective nature, the taste is quite a fair index of astringency. In a 0.9 per cent. NaCl solution of tannin, the threshold of taste and of protein precipitation coincide closely, as shown by the following.

Per Cent. of Tannin	Taste	Precipitation of Serum
0.0625	Slightly astringent	Turbidity
0.03125	Slightly astringent	Doubtful
0.015625	Not astringent	None

The filtered digests made of 2.5 per cent. of tannalbin, tannigen and tannoform, respectively, were tested with the following essentially negative results, which agree throughout with the serum precipitation.

NaCl: Taste not astringent with any.

Pepsin-HCl, neutralized: Doubtful with tannalbin; not astringent with the others.

Trypsin-Bicarb, neutralized: Doubtful with all.

Astringent Taste of the Dry Powders.—The taste of the dry powders seems to be a more delicate test of astringency than the taste of the solutions. A penknife pointful of the powder is placed on the tip of the tongue and chewed. With this test, the following results were obtained, in ascending order of astringency. The numbers in parenthesis are the code numbers of the specimens.

Tannopin (12 and D): Entirely nonastringent.

Tannigen (1, 6, 13, 25) and Acetannal: Acid; then slightly astringent.

Gallogen (11, 19 and 22): First slightly salty and acid; then slightly astringent.

Tannalbin (A, 2, 9, 14) and Tannin Albuminate exs. Merck (17, 24): Slightly astringent.

Tannismuth (10, 20): Quite astringent from start.

Protan (5, 7, 16, 23): Quite astringent from start.

Tannoform (3, 4, 8, 15): Quite astringent from start.

Tannigen (exceptional No. 21): Highly astringent. Evidently decomposed.

The Frog-Lung Method of Estimating Astringency.—Dreser suggested the extensibility of the frog lung as an index of astringency. It can be measured by tying the sacular lung over a cannula. This is attached to a tube, which is immersed to a given depth in a cylinder of water. The level to which the water rises in the tube is proportional to the expansion of the lung. It is diminished by laying the lung into astringents. The details are described in Sollmann, Laboratory Guide, page 159. Comparisons may be made by working under standard conditions.

The following experiments were carried out by Mr. G. E. Richardson. For each reading, the tube was immersed in the cylinder for one-half minute. The lung was then laid into the astringent solutions for the periods shown in the experiments. All solutions were made up with 0.6 per cent. NaCl. Acid and bicarbonate both corrode the lungs; so that the digests were neutralized before testing.

Results.—These are shown in Table 4. The figures correspond to the differences in per cent. of the original reading in the same experiment. "+" indicates increase of extensibility, "—" decrease of extensibility, i.e., astringent action. Only the median figures for all experiments are reproduced.

It may be noted that in saline alone, the extensibility of the lung tends to increase (till about forty-five minutes); but in all of the astringents, it diminishes. Typical astringents, viz., copper sulphate and tannic acid, cause a rapid and profound decrease of extension. This tanning action begins at once and is well advanced in five minutes. In the case of tannic acid, it is usually completed in one-half hour. The effect is proportional to the concentration.

As to the masked tannin compounds, neutral saline extracts contain sufficient tannin to be very distinctly astringent. Extracts made with artificial gastric juice are still more astringent. The results for alkaline tryptic digests are probably unreliable.

The results leave no doubt that these preparations are astringent for tissues, and as potent as tannin solutions of 0.5 per cent. or stronger. In vivo, the degree of astringency would probably be less,

because of the relatively slow solubility. On the other hand, this would prolong the action farther into the intestines.

The lung test is evidently a good qualitative and rough quantitative test of astringency, but it is much less convenient than the protein precipitation test, and is unnecessary except as a control for special questions.

TABLE 4.—MEDIAN EFFECTS OF ASTRINGENTS ON FROG-LUNG

	Approximate Time of Immersion of Lung in Astringent			
A: Astringents in 0.6% NaCl	5 Minutes	15 Minutes	30 Minutes	¾ to 1¼ Hours
None (i. e., NaCl alone)	....	+ 4	+26	+28
$CuSO_4$ 0.1%	—55	—58	—74	
Tannic acid 2.5%	....	—59	—70	—87
Tannic acid 1%	—11	—23	—48	—48
Tannic acid 0.5%	....	—30	—35	—62
Tannic acid 0.25%	....	—18½	—23	—33
Tannalbin, Spec. 2, 2.5%	....	—46	—58	—64
Tannigen, Spec. 1, 2.5%	....	—22	—40	—40
Tannoform, Spec. 3, 2.5%	....	—30	—24	—52
Tannalbin, Spec. A, 1%	....	—15		
Tannigen, Spec. B, 1%	....	—20		
Tannoform, Spec. C, 1%	....	— 3	—14	—26
Tannopin, Spec. D, 1%	....	— 5	—10	—18
B: Acid-pepsin digests, neutralized to litmus:				
None (i. e., NaCl alone)	....	— 7	—19	—30
Tannin, 2.5%	....	—75	—78	
Tannalbin, Spec. 2, 2.5%	....	—37	—48	—67
Tannigen, Spec. 1, 2.5%	....	—33	—55	—77
Tannoform, Spec. 3, 2.5%	....	—40	—84	—84
C: Bicarbonate-trypsin digests, neutralized to litmus:				
None (i. e., NaCl alone)	....	+ 5	+ 5	+ 5
Tannin, 2.5%	....	—13	—63	—63
Tannalbin, Spec. 2, 2.5%	....	— 4	— 3	—22
Tannigen, Spec. 1, 2.5%	....	+ 1	+ 5	— 2
Tannoform, Spec. 3, 2.5%	....	+ 1	+ 1	— 5

TANNIN PREPARATIONS ON NORMAL STOOLS

Some experiments were performed on a normal human subject to note whether tannin astringents have a constipative action on normal stools. The experiments were made as a continuous series, and the results are given in the sequence in which they were obtained.

	Average Number of Stools of Day
Fore period, 4 days	1
Gallic acid, 1 gm. in milk after lunch	
Two following days	1
Tannin, 0.5 gm. in milk, after lunch	
Next day	1
Tannin, 2 gm. in tea, with lunch	
Next day	1
Second to ninth day	1½
Tenth and eleventh day	1
Catechu, 3 gm., powdered, in tea, with lunch	
Next day	1
Catechu, 10 gm., powdered, in tea, with lunch	
Next day	1
Second to seventh day	2
Eighth and ninth day	1½
Tannalbin (A) 10 gm., dry, after lunch	
Next day	2
Second and third day	1

It is very evident that there is no effect on the number of normal stools; nor was the normal formed consistence affected. The experiments were not continued further. Determinations of the moist or dry weight might have been more positive, but it seemed improbable, in view of these negative results, that they would jusify that trouble of exact control of the diet.

The astringent effects in the mouth and esophagus were interesting, as to their intensity and especially as to their duration. The suppression of secretion persisted over half an hour, indicating that the tannin-tissue precipitations are but slowly dissolved. The details are as follows:

Tannin, 2 gm., in 0.5 liter of tea, swallowed slowly with lunch. Taste very distinctly astringent; throat dry and swallowing difficult. The dysphagia lasts about half an hour. During the afternoon there is some heartburn and heavy gastric sensation.

Tannin, 0.5 gm. in milk: Effects not marked.

Gallic acid, 1 gm. in milk: Taste slightly bitter; not astrigent, except the dregs.

Catechu, powder, 3 gm. and 10 gm., in tea: Astringent taste. Throat slightly dry with the smaller dose, distinctly dry with the larger dose.

DISCUSSION OF THE RESULTS WITH THE INDIVIDUAL DRUGS

In this section, there will be discussed the results obtained on the individual drugs, with the various methods. The percentages of the drugs are stated in terms of the final mixtures. Unless otherwise explained, "neutral saline" stands for 0.9 per cent. NaCl, except in the frog-lung experiment, where 0.6 per cent. NaCl was used. "Acid digest" means the filtrate after about four hours' digestion with saline containing N/15 HCl, i.e., about the acidity of gastric juice. "Bicarbonate digest" means 0.5 per cent. sodium bicarbonate under analogous conditions; i.e., the reaction of the intestinal juice. "Neutral" means that the filtrate was neutralized to methyl orange; except with "sodium carbonate," which was digested with 1 per cent. of the carbonate and the filtrate neutralized to litmus. The further details were described under the methods.

Tannic Acid.—This serves as a standard of comparison for the others.

Albumin Precipitation: When 2 per cent. of tannin was added to 10 per cent. moist egg white, the albumen was completely precipitated in 1 per cent. sodium bicarbonate solution; 63 per cent. was precipitated in N/15 HCl. Precipitation therefore occurs at full gastric acidity, but is less complete than when the solution is less acid.

Tannin and Serum.—Neutral Saline Tannin Digest: Precipitation of serum begins with mixtures containing 0.015625 per cent. of

tannin, and is complete with those containing 0.125 per cent. of tannin. The precipitate is much greater than with any of the tannin compounds.

The following serum experiments all refer to mixtures containing 0.125 per cent. of tannin.

Acid Digest: Before neutralization, this digest produced only turbidity, when added to serum. After neutralization, the turbidity was very much heavier.

Acid-pepsin Digest: Before neutralization, the digest did not precipitate serum; but after neutralization, it gave a heavy precipitate. The failure to produce turbidity in acid reaction is probably due to the binding of some of the tannin by the protein of the pepsin.

Bicarbonate Digest: Before neutralization, serum produced only turbidity; after neutralization, it gave a strong precipitation.

Tannin Acid Destruction by Sodium Bicarbonate: If the tannin is digested for several hours at 40 C. with 1 per cent. sodium bicarbonate, and then neutralized, distinctly higher concentrations are needed for precipitation, than with neutral saline digests, viz., 0.0625 and 0.5 per cent. for beginning and complete precipitation, instead of 0.015625 and 0.125 per cent., respectively. This indicates that some of the tannin is destroyed in alkaline digestion experiments.

Trypsin-Bicarbonate Digest: This behaved essentially like the bicarbonate, both before and after neutralization. The precipitate in the nuetral solution was not quite as heavy, presumably because some of the tannin had been precipitated by the protein of the trypsin.

Bile-Bicarbonate Digest: This gave precipitation even without neutralization.

Sodium Carbonate Digest: After neutralization, this gave a precipitate showing that the tannin had not been destroyed.

Antihemolytic Efficiency of Tannin Digest.—Saline Solutions: A concentration of 0.125 per cent. protects completely against water laking. With 0.0625 per cent. the protection is partial. With 0.03125 per cent. there is practically no protection. (Kobert found complete agglutination with 1: 20,000.)

Acid Digest and Acid-Ppsin Digest: 0.1 per cent. tannin, after neutralization protects completely.

Bicarbonate Digest: Before neutralization, 0.125 per cent. protects only incompletely. After neutralization, 0.1 per cent. gives complete protection.

Trypsin-Bicarbonate Digest: Before neutralization, no protection with 0.25 per cent.; after neutralization, complete protection with 0.25 per cent.

Sodium Carbonate Digest: After neutralization, 0.25 per cent. gives complete protection.

Astringent Taste of Saline Tannin Solution.—Slight astringency is noted with 0.03125 per cent.; none with 0.015625 per cent.

Tannin on Lung Elasticity.—The expansion is diminished 23 per cent. by 0.25 per cent. tannin, and 74 per cent. by 2.5 per cent. tannic acid. Practically the same results are obtained with the neutralized acid-digest.

Summary of Tannic Acid.—The various methods of testing astringency confirm that tannin is astringent for the whole range of reactions that exist in the digestive tract; but that the precipitation, and therefore the astringency are less efficient at the extremes of reaction, the extreme acidity or the gastric juice being more unfavorable than the alkalinity of the intestinal juice.

The individual methods compare as to sensitiveness as follows:

	Beginning Reaction Per Cent.	Marked Reaction Per Cent.	Complete Reaction Per Cent.
Per cent. of tannin needed for:			
Serum precipitation	0.015625		0.125
Antihemolysis	0.0625-0.03125		0.125
Taste	0.015625-0.03125		
Lung expansion		0.25	

Acid Digest: Before neutralization, these produce only slight precipitation of serum and incomplete precipitation of egg albumin.

Acid Digest After Neutralization: These produce full strength reactions by all the methods.

Pepsin-Acid Digests: These are slightly weaker than the simple acid digests, presumably because a part of the tannin had been bound by the protein of the pepsin.

Bicarbonate Digests Before Neutralization: These give complete precipitation of the egg albumin, but only turbidity with serum, and incomplete protection on laking.

Bicarbonate, Trypsin-Bicarbonate, and Bile-Bicarbonate Digests After Neutralization: These give a strong precipitate with serums and complete protection against laking. However, some of the tannic acid is destroyed during the alkaline digestion.

Sodium Carbonate Digests: After neutralization, these precipitate serum and protect against hemolysis.

Gallic Acid.—It was pointed out in the previous article that this precipitates proteins in buffer solutions essentially like a weak tannic acid solution; presumably because it is contaminated with a trace of this substance.

Serum experiments with galic acid: The present series showed no precipitation in the natural (acid) reaction of 0.5 to 4 per cent. mixtures. After neutralization, slight turbidity occurred; also in neutralized acid, acid-pepsin, bicarbonate, and bicarbonate trypsin digests, containing 0.25 per cent of the gallic acid.

Gallic acid and other proteins: Egg-white solution, 1:10, was rendered only slightly turbid. The turbidity was not increased by sodium bicarbonate, nor by acacia. The latter experiment was made to test an old statement, attributed by Headland to Pelletier.

Dog-serum gave a large precpitate, which was not increased by sodium bicarbonate.

Antihemolytic efficiency of gallic acid: Saline solutions of gallic acid are directly hemolytic, doubtless though the acidity. After neutralization, there is no direct laking, nor is there any protection against water-laking. The same total inefficiency was found for neutralized digests with acid, pepsin acid, bicarbonate, and trypsin-bicarbonate. (Kobert found that saline solutions of gallic acid had one sixtieth of the agglutinating efficiency of tannic acid.)

Summary of gallic acid: Gallic acid behaves like a very dilute tannic acid, i.e., a 0.5 per cent. solution of gallic acid is about equivalent to 0.03 per cent, of tannic acid. It would have little or no practical value as an astringent.

TYPES OF MASKED TANNIC COMPOUNDS

These fall into several groups:

Astringent Extracts.—It has been generally supposed that the colloidal extractives of crude drugs and extracts would slow the access and action of their tannin, so as to avoid gastric effects, and would therefore prolong the action into the intestines. Direct experiments [2] show, however, that solutions of these drugs precipitate like tannin itself. Delay in the action could occur by delay in the solution. Such delay would be probable in the case of the powdered drugs, and perhaps with the solid extracts; but it would not apply to solutions, infusions, etc.

It is conceivable that the astringent action would be slowed by the colloids impeding the access of the tannin of the mucosa.

Tannin-Protein Compounds

The precipitation of proteins by tannin has been utilized to render the tannin itself more or less insoluble, with the intention of checking of delaying its action. As has been explained, such precipitates may be redissolved under suitable conditions in the digestive tract, and thus reacquire astringent properties.

Freshly Precipitated Tannin Albuminate.—This was introduced by Lewin, 1881, and again endorsed by him in 1904. It would have relatively little effect on the stomach; although it would be somewhat astringent at the optimal reaction.[2] It would become astringent in the duodenum, but could probably not act much beyond this.

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2. Sollmann, Torald: Jour. Pharmacol. & Exper. Therap. **16**:49 (Aug.) 1920. Vierordt, O.: Deutsch. med. Wchnschr., 1896, No. 25, p. 389.

Lewin employed a mixture of 2 to 5 gm. of tannic acid in 150 c.c. of water, to which the white of an egg is added, gradually and with constant stirring.

Coagulated Tannin Albuminate.—This was introduced by Gottlieb, 1896, and was marketed under the name Tannalbin. New and non-official Remedies (N. N. R.) has adopted the group-name "Albutannin" for all the commercial brands. These differ, however, in solubility.

Tannalbin is a heat-coagulated tannin albuminate, made by precipitating egg albumin with tannin, washing the precipitate, and drying for six hours at 126°C. It should contain about 50 per cent. of tannin, and is claimed to be practically insoluble in water; partly soluble in dilute acid, and slowly soluble in alkaline fluids, with liberation of its constituents.

Clinical trials were conducted by O. Vierordt, 1896. He considers it better than tannigen: The taste is but little astringent, even when kept in the mouth for a considerable time. It was satisfactorily efficient in acute and chronic diarrheas. In colitis, it diminished the mucus

TABLE 5.—SOLUBILITY OF TANNIN PROTEIN COMPOUNDS

	Median and Range of Percentage of the Compound Dissolved by				
	Water	Acid	Acid Pepsin	Bicarbonate	Bicarbonate Trypsin
Tannalbin, claimed.	Practically insoluble		Partly soluble	50 per cent. slowly soluble, with cleavage	
Tannalbin, found...	20 (18-21)	34	79 (60-86)	49 (42-52)	59 (58-68)
Merck.....	21	32	72 (72-73)	41	48 (45-51)
Calco and M. C. W	16 (15-16)		96 (95-96)	33 (31-34)	82 (77-86)
Protan, found.....	16 (14-16)	35 (20-38)	97 (73-81)	90	94 (81-98
Protan, claimed....	Insoluble	Insoluble		Soluble	

materially. The action was uniform. There was no gastric disturbance. It did not influence healthy stools.

Subsequent clinical trials have been satisfactory, without establishing any definite superiority over the other "insoluble" tannin preparations.

Solubility of Tannin-Protein Compounds.—The determinations of Leech, conducted under conditions approximating those of the body, differ radically from the advertised claims.

The investigation included specimens of the original "Tannalbin" (Knoll); of "Tannin Albuminate Exsicated" (Merck); a specimen each of Calco and M. C. W. (Mallinchrodt) "Albutannin"; and also specimens of "Protan" (Mulford), an analogous tannin casein compound.

The median solubility is shown in Table 5.

All dissolve to a considerable degree, though slowly, in water. More dissolves in dilute acid, and still more in the presence of pepsin. Bicarbonate generally dissolves but little more than acid, and less than acid-pepsin. Even trypsin bicarbonate tends to dissolve less than acid-pepsin.

Comparing the different compounds, it is seen that the Merck product agrees practically with the tannalbin in showing a rather limited solubility, but greater in acid pepsin than in bicarbonate trypsin. The Calco and M. C. W. are more completely soluble in both media; this may be rather a disadvantage, because it would exhaust the tannin in the upper intestine. Protan differs from the albutannins in its high solubility in alkali, even without trypsin. This also is probably rather disadvantageous.

Speed of solution of the tannin-protein compounds: This is usually a rather slow process. This slowness is quite as important as

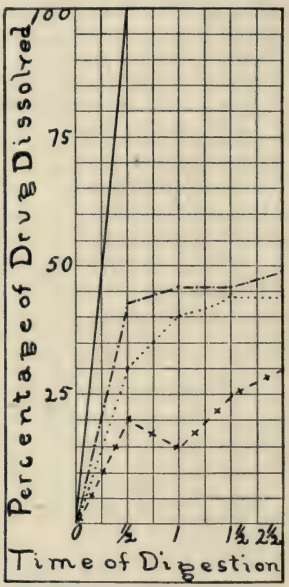

Fig. 1.—Time factor in solution of tannin-protein compounds. Digestion with acid-pepsin. ——— = protan; ..... = tannin albuminate, Merck; -.-.-. = tannalbin; + — + — + = gallogen.

is the absolute solubility, in determining the site of the astringent action. The results of Leech are shown as curves in Figures 1 and 2. In both the acid-pepsin and alkaline-pancreatin digests, the solution is at first fairly rapid. However, with tannalbin and the Merck product, considerable further solution occurs during the next hour, or longer. This would prolong the action of the tannin for considerable distances into the intestine. With protan the solution in alkaline pancreatin is completed so promptly that the conditions for prolonged action would be unfavorable.

Is the protein-tannin compound dissolved unchanged? Leech found the nitrogen percentage of the undissolved residue of the

Merck product and of Protan after various periods of peptic and pancreatic digestion practically unchanged. This indicates that at least the major part of the tannin is really combined with the protein.

Since "peptone" reacts with tannin practically like native proteins (Sollmann; Brühl; Hammersten), the greater solubility of the tannin albuminates in the presence of ferments is probably due merely to the fact that the fements facilitate the solution of the heat-coagulated protein-tannin compounds.

Solubility standards: The variation in the solubility of the tannin protein compounds is so wide that it is likely to affect their therapeutic action. It seems better to restrict the solubility in dilute acid to from 25 to 35 per cent. and in bicarbonate to from 35 to 55 per cent., as determined by the methods employed by Leech. Compounds

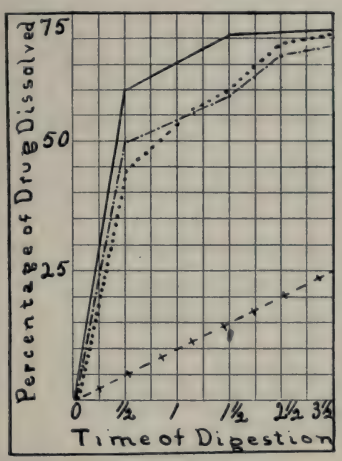

Fig. 2.—Time factor in solution of tannin-protein compounds. Digestion with bicarbonate trypsin. ——— = protan; ..... = tannin albuminate, Merck, -.-.-.- = tannalbin; + — + — + = gallogen.

that have different solution should not be classed as identical with tannalbin. Protan should have somewhat different standards.

Egg Albumin Precipitation by Tannin Albuminate.—The percentage of the albumin precipiated by 2 per cent. solutions of the astringents was as follows:

	HCl n/15	Sodium Bicarbonate 1 Per Cent.
Tannalbin, Knoll (Specimen 2)	26	76
Albutannin, Merck (Specimen 24)	26	39(?)
Albutannin, Calco	35	97
Tannic acid	63	100

It is seen that the compounds precipitate albumin both in acid and alkaline media, although not quite as much as tannin. Evidently,

the tannin albuminates are actually capable of precipitating further quantities of albumin under suitable conditions. The precipitation is more effective in the alkaline reaction.

The relative precipitant efficiency of the different compounds is proportional to their own solubility, as judged by the amount of nitrogen dissolved in the media, in the absence of added proteins. This was as follows, for the filtrates from 2 per cent. suspensions, in terms of milligrams of nitrogen per hundred mg. of drug:

	HCl n/15	Sodium Bicarbonate 1 Per Cent.
Knoll	2.0	1.6
Merck	2.0	0(?)
Calco	2.3	3.8

Tannin Proteinates and Serum.—The digests were made with 2 or 2.5 per cent. of the tannin preparations. The filtrates of these were diluted with equal volumes of the serum solutions. The results are shown in Table 6.

It is very evident that the phenomena are quite different for protan and tannalbin.

Protan precipitates almost as much as uncombined tannin, except in the trypsin digest. In this case, the tannin of the protan was probably used up during the digestion in precipitating the protein of

TABLE 6.—SERUM PRECIPITATION BY TANNIN PROTEIN COMPOUNDS

Menstruums	Tannalbin, Specimen 9, 2 Per Cent.	Tannalbin, Specimen 2, 2.5 Per Cent.	Protan (Caseinate), Specimen 5, 2 Per Cent.
Neutral saline	No precipitate	No precipitate	Precipitate
Hydrochloric, acid	No precipitate		Precipitate
Hydrochloric, then neutralized	Slight turbidity		
Pepsin, acid	No precipitate		
Pepsin, acid, then neutralized	Precipitate		Heavy precipitate
Bicarbonate, alkaline	Turbidity		Precipitate
Trypsin, alkaline	Turbidity		
Trypsin, neutralized	No precipitate	No precipitate	Turbidity
Bile, alkaline, neutralized	No precipitate		
Carbonate, alkaline, neutralized	Slight turbidity		

the trypsin. The caseinate therefore protects but little against the direct effects of tannin.

Tannalbin is only slightly astringent in any of the digests. The largest precipitation is given by the neutralized acid-pepsin digest; turbidity is produced by the neutralized acid digest, and by the alkaline bicarbonate and trypsin digests. No precipitate is given by the unneutralized acid digests; or by the neutral saline digests.

Antihemolytic Efficiency of Tannin Proteinates.—The results are shown in Table 7. "Laking" is equivalent to absence of astringency.

The results again show the radical difference between the two compounds: Protan protects completely against laking under all conditions, i.e., its astringency is essentially equal to that of unbound tannin; Tannalbin is much less active. However, efficient protection, i.e., astringency, is exerted by the neutralized acid digest, by the neutral saline digest, and to a slighter degree by the alkaline digests. This agrees with the serum test, except that the hemolysis method shows astringency even for neutral saline digests, but only for concentrations above 1 per cent.

Kobert found the agglutination efficiency of neutral solutions of tannalbin to be one twentieth of that of tannic acid.

Astringent Taste of Solutions.—Filtrates of 2.5 per cent. digests of tannalbin have little or no astringent taste. The neutral saline digest was nonastringent; the neutralized acid-pepsin and bicarbonate-trypsin digests were doubtful.

TABLE 7.—TANNIN PROTEINATES ON HEMOLYSIS

Menstruums	Tannalbin, Specimen 2, 2 Per Cent.	Tannalbin, Specimen 9, 2 Per Cent.	Protan, Specimen 5, 2 Per Cent.
Neutral saline, 2 per cent. suspension	.....	Laked	
Neutral saline, filtrates, 2 and 1 per cent.	No Laking	.....	No laking
Neutral saline, filtrates, 0.5 per cent....	Doubtful		
Neutral saline, filtrates, 0.25 per cent...	Laked		
Acid, neutralized, 2 per cent.....	No laking		
Acid, neutralized, 0.5 per cent.....	Laked	.....	No laking
Acid, pepsin, neutralized.....	.....	.....	No laking
Bicarbonate, alkaline, 2 per cent.....	Doubtful	Laked	No laking
Bicarbonate, alkaline, 0.5 per cent.....	Doubtful	.....	No laking
Bicarbonate, neutralized.....	.....	.....	No laking
Alkaline trypsin, neutralized.....	.....	.....	No laking
Carbonate, neutralized, 2 per cent.....	.....	No laking	

Astringent Taste of the Powders.—All samples of tannalbin taste slightly astringent when chewed; more so than tannigen, and less than protan or tannoform.

All samples of Protan are distinctly astringent from the start, more so than any other masked tannin compounds except tannoform.

Tannalbin on Lung Elasticity.—The extension of the lungs is diminished by the neutral saline and by the neutralized acid and neutralized trypsin bicarbonate digests, as follows:

Neutral saline: 2.5 per cent., Specimen 2, about like 1 per cent. tannin.

Neutral saline: 1 per cent., Specimen A, less than 0.25 per cent. tannin.

Neutralized acid: 2.5 per cent., Specimen 2, about like 1 per cent. tannin.

Neutralized trypsin bicarbonate: 2.5 per cent., Specimen 2, about like 0.25 per cent, tannin.

Summary of Tannin-Protein Compounds.—These do not comply with the claimed solubility. In fact, they are slowly and incompletely

dissolved at all physiologic reactions, especially in the presence of the digestive ferments. The specimens, however, differ materially, especially in their behavior toward trypsin bicarbonate, the American products being more soluble than the original.

Limited, and especially slow, solubility would probably extend the astringent action to lower levels of the intestine than the more rapidly soluble products. The rapid and complete solubility of protan is therefore probably undesirable if the action is to extend beyond the duodenum.

The commercial tannin albuminate is capable of precipitating further quantities of albumin, even at the extreme reactions of the stomach and intestine. The precipitation is proportional to the solubility of the compounds. Alkalinity interferes less with the precipitation than the full acidity of the gastric juice.

The various tests of astringency show *protan* solution to act essentially like free tannin. Its slower solubility would, however, mitigate its action on the stomach, and extend it somewhat into the intestines.

Tannin albuminates are very mildly astringent, by all the tests. The largest precipitation is given by the neutralized acid-pepsin digests; then come the neutralized bicarb-trypsin digests; and then the neutral digests. Unneutralized acid digests are not precipitant.

The results indicate the possibility of mild astringency in the mouth, which is confirmed by the taste. Only slight astringency would occur in the stomach until digestion advanced, and then it would be largely suspended by the strongly acid reaction. Considerable astringency would develop in the duodenum when the acidity is reduced. Some astringency would continue into the lower intestines, following the cleavage of the undissolved proteinate.

These properties would all be therapeutically desirable.

TANNIC ACETIC ESTER

Acetic acid esters of tannin were introduced into therapeutics by Hans H. Meyer, 1894, under the name *Tannigen*. The common name acetannin was introduced into N. N. R. to cover all the commercial brands.

Claims.—Although the discovery of tannic acetic esters is universally credited to Hans Meyer, then of Marburg, the official United States patent application of September, 1894 (522,731) claims the discovery of the substance and of its therapeutic qualities for one "Jacob Meyer, chemist and doctor of philosophy," then of Frankfort-on-the-Main; and the patent was granted to him and his representatives.

Tannigen and the various brands of acetannin are said to be prepared by the methods described in this original patent; that is

by heating tannin and acetic anhydrid in the presence of glacial acetic acid. Here, however, another discrepancy occurs. The original patent describes the product of this process as "a mixture of monoacetylated and diacetylated gallic anhydrides," that is of monoacetyc and diacetyl tannins. The commercial products, however, are sold under the direct or implied claim that they are a definite compound, diacetyl tannin, $C_{14}H_8(CH_3CO)_2O_9$; and not as indefinite mixtures. The varying properties of different specimens indicate that the information of the original patent was correct; that is, that the commercial article is a mixture and not a definite chemical.

Tannigen was described by H. Meyer as practically insoluble in water and therefore tasteless. It is dissolved by alkaline mediums (even sodium borate). On boiling, or on prolonged standing of the alkaline solutions, is is saponified into acetic and gallic acid; while ammonia produces acetic and tannic acids. Fresh solutions in sodium phosphate act as astringents; they precipitate glue and albumin and inhibit the secretion of frog's skin. The precipitates are redissolved by stronger alkalis, even borate; and these prevent the astringent action on the frog's skin. Apparently, therefore, the solutions contain free tannin, and this is probably the ordinary primary cleavage product will all dilute alkalis. E. Rost, 1897, proved that in the intestines tannigen is changed into tannic acid, and not into gallic acid.

On administering it to animals, H. Meyer found the stools more solid. The feces contained tannin, even after small doses, so that the action would extend to the end of the intestinal tract. The urine did not contain gallic acid, so that the absorption must have been slight.

Tannigen was subject to clinical trial by Friedrich Müller, 1894. He found it free from side effects, and efficient in chronic diarrheas of various origin, especially tuberculous. That is, it generally diminished the number and increased the consistence of the stools. In acute diarrhea, it was difficult to draw conclusions because of the uncertainty of spontaneous cessation.

O. Vierordt, 1896, an advocate of tannalbin, was less enthusiastic about tannigen. He claimed that tannigen is somewhat astringent in the mouth, and therefore also in the stomach. Long continued use may disturb the appetite. Its intestinal action is often not sufficiently powerful.

The later trend of the literature seems to be generally favorable, although not very critical. It is fairly well reflected in the following quotation from the circular of the manufacturers:

Professor C. A. Ewald, Berlin: The results, according to my experience and that of Biedert, Escherich, Kunkel, Hock and others, are nearly without exception prompt, and the drug is certainly more reliable than rhatany, catechu, hematoxylon, and other astringents.—*Therap. Wchnschr.*

The use of acetannin therefore depends on the fact that it is almost insoluble, but that it is hydrolyzed under certain conditions, with the liberation of tannin. The rapidity of hydrolysis varies with the conditions, and this probably explains some of the contradictory results. The ester is soluble in dilute alkali, and this doubtless favors hydrolysis; but the conditions of solution and hydrolysis are distinct. This has not been sufficiently clearly realized in the literature of tannigen. It will be shown that dissolved but undissociated acetyl tannin does not precipitate proteins.

Some hydrolysis appears to occur even in the powdered drugs; for each of four specimens of tannigen had a well-marked acetic odor.

Solubility of Acetannin.—The advertising matter of tannigen claims that it is insoluble in dilute acids and cold water, but dissolves in dilute alkalis.

The solubility, as estimated by Leech, showed considerable difference from the claims, and between the individual specimens, as will be seen from Table 8.

TABLE 8.—SOLUBILITY OF TANNIN ACETIC ESTERS

	Median and Range of Percentage of the Compound Dissolved by				
	Water	Acid	Acid Pepsin	Bicarbonate	Bicarbonate Trypsin
Tannigen, claimed.	Insoluble	Insoluble	Insoluble	Soluble	Soluble
Tannigen, found...	8.5 (8.5-17.2)		6.1 (3.8-17.4)	75 (31-100)	66 (32-100)
Acetannal Calco..	6.8	6.2	5.5	91	87

All the specimens are somewhat soluble in water and equally so in dilute acid. This has probably no practical importance, except as a test of quality.

The apparent difference in the alkali-solubility is important. Only two of the four preparations are completely or nearly completely soluble. The other specimens, both of the original German manufacture, are evidently contaminated with large quantities of insoluble by-products, which are probably to be considered as inert ballast, from the therapeutic standpoint. It is desirable that such specimens should be excluded by appropriate solubility tests.

The solubility appears slightly lower in the trypsin solution. This is doubtless due to the precipitation of some of the protein of the pancreas preparation.

Hydrolysis of Acetannin.—Leech noted a further point that is probably of material therapeutic importance; namely, that the hydrolysis of the dissolved ester proceeds quite slowly, when digested with 1 per cent. bicarbonate at 40 C. The presence of nonhydrolyzed esters is shown by precipitation on the addition of acid. The results for three specimens are shown in curves in Figures 3. Theoretically, 1 gm.

of di-acetyl tannin, when completely hydrolyzed, should require 7.8 c.c. of normal alkali. The astringency was estimated by the precipitation of egg albumin solution.

The figure shows that the hydrolysis is only half completed in one-half hour, and reaches its acme in one or two hours. This slow liberation is doubtless a therapeutic advantage, in that it prolongs the action further into the intestines. The figure also shows that the precipitation of albumin is parallel to the hydrolysis; i.e., the ester

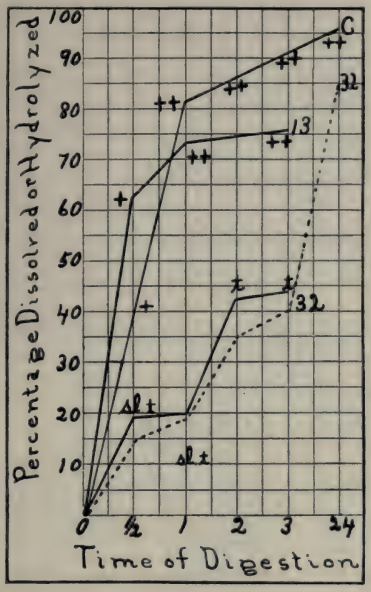

FIG. 3.—Time factor in hydrolysis of acetannins. The specimens are digested with 1 per cent. sodium bicarbonate at 40 C. Solution (- - - - -) is estimated by the weight of the undissolved residue. Hydrolysis (————) is determined by titration of the liberated acid. The precipitation of protein is shown by the symbols sl.t. = slight turbidity; t = turbidity; + = precipitate; ++ = heavy precipitate. The specimens used were: No. C., acetannin Calco; No. 13, tannigen Bayer; No. 32, tannigen Winthrop.

as such is nonastringent. That part which escapes hydrolysis is therefore inert ballast. The figure shows that this varies, in the different specimens, at the end of three hours, from 10 to 60 per cent. Tests should be devised to limit this inert material to a minimum.

Egg Albumin Precipitation by Acetannin.—The percentage of albumin precipitated by 2 per cent. solutions was as follows:

	N/15	Sodium Bicarbonate 1 Per Cent.
Tannigen (Specimen 1)	0	62
Acetannal	0	96

Acetannin does not precipitate in the acid medium, differing in this from the albutannins. This would be a therapeutic advantage. The precipitation in the alkaline medium is effective; the German specimen, however, being inferior to that of American manufacturers.

Serum Precipitation by Acetannins.—The digests were made with 2 or 2.5 per cent. of the tannin preparations. The filtrates of these were diluted with equal volumes of the serum solutions. The results are shown in Table 9.

TABLE 9.—SERUM PRECIPITATION BY ACETANNINS

Menstruums	Tannalbin, Specimen 6, 2 Per Cent.	Tannalbin, Specimen 1, 2.5 Per Cent.
Neutral saline	No precipitate	No precipitate
Hydrochloric, acid	No precipitate	
Hydrochloric, neutralized	Turbidity	
Pepsin, acid	No precipitate	
Pepsin, neutralized	Turbidity	
Bicarb, alkaline	Turbidity	
Trypsin, alkaline	No precipitate	
Bile, alkaline	Turbidity	
Carbonate, neutralized	No precipitate	

The acetannins again fail to precipitate at acid reaction. Turbidity, that is, slight astringent effect, is given by the alkaline extracts, even at the alkaline reaction; and by the neutralized acid extracts. Neutral saline extract does not give turbidity.

These results agree essentially with those of the albutamines.

Antihemolytic Efficiency of Tannigen.—The results are shown in Table 10. "Laking" is equivalent to absence of astringency.

TABLE 10.—TANNIGEN ON HEMOLYSIS

Menstruums	Specimen 1 2 Per Cent.	Specimen 6 2 Per Cent.
Neutral saline	Partly laked	Laked
Hydrochloric, neutralized	Not laked	
Bicarbonate, alkaline	Not laked	Laked
Trypsin, alkaline		Laked
Bicarbonate, neutralized	Not laked	
Carbonate, neutralized		Not laked

By this test, there appears considerable difference between the two specimens.

Specimen 6 was not astringent in neutral saline, or neutralized alkaline or trypsin digests; but only as the neutralized carbonate digest.

Specimen 1 gave complete protection for the alkaline digest, with or without neutralization, and with the neutralized hydrochlorid digest; and partial protection with the saline digest.

The results illustrate the variable activity of the specimens. Specimen 6 would be very little astringent (it gave some turbidity with the serum test). Specimen 1 behaves essentially like the albutannins.

Kobert found the agglutination efficiency of neutral solutions one-fourth that of tannin; a result that could only be anticipated with a decomposed product.

Astringent Tests of Tannigen Solutions.—Filtrates of 2.5 per cent. saline and of neutralized pepsin-hydrochloric digests of tannigen had no astringent taste. The taste of neutralized trypsin-bicarbonate digest was doubtful. The astringent action in any case therefore cannot be strong.

Taste of Acetannin in Powder.—The advertising claims that tannigen is tasteless. This is not strictly true of any specimen, and far from true in some cases. Most specimens have an acid taste, which becomes somewhat astringent on chewing. The astringency is, however, very slight, tannopin being the only masked tannin compound of those examined that tastes less astringent than acetannin.

One specimen, however (German tannigen, specimen 21), was very highly astringent; more so than any other specimen of any masked tannin compound. This again illustrates the unfortunate variability of the commercial drug.

Tannigen on Lung Elasticity.—The extension of the lungs is diminished by the neutral saline and by the neutralized pepsin hydrochloric digests, on the average about as much as by albutannin. The results were as follows:

Neutral saline, 1 per cent., Specimen B: like 0.25 per cent. tannin, more than tannalbin, less than tannoform.

Neutral saline, 2.5 per cent., Specimen 1: Like 0.5 per cent. tannin, less than tannalbin, more than tannoform.

Pepsin hydrochloric, neutralized, 2.5 per cent., Specimen 1: Like 2.5 per cent. tannin; more than tannalbin or tannoform.

Trypsin bicarbonate neutralized 2.5 per cent., Specimen 1: Very little astringency; evidently some technical error, perhaps combination with the protection of the trypsin.

Summary of Tannic Acetic Esters.—The commercial acetannin, and especially the original "tannigens" are variable mixtures. Some approach very closely the ideal requirements; some consist mainly of insoluble and inert ballast, and some contain so much free tannin that they could not be classed as "masked" tannin compounds.

The best specimens are very slightly soluble in neutral and acid fluids, although they do have a slight astringent action even under these conditions. They dissolve in weakly alkaline fluids, but the solutions are not directly astringent. They become astringent as the ester is hydrolyzed, which occurs gradually and extends over two hours, under conditions approximating those of the intestines.

As compared with the tannin proteinates, they would be somewhat less astringent in the stomach and upper intestines, and would develop their action gradually and progressively in the lower intestines. The astringency would always be mild.

Their special field, therefore, would appear to be when the astringent action is desired in the lower intestines. However, they do not deserve full confidence until the commercial products are more uniform. The high quality of some of the samples makes the outlook hopeful.

TANNOFORM

Composition and Solubility.—Tannoform is said to be a condensation product of tannic acid and formaldehyd, $CH_2(C_{14}H_9O_9)_2$. It is claimed to be insoluble in water or dilute acid, and soluble in alkalis, and it is supposed to act in alkaline solutions both as tannin and as formaldehyd.

The actual solubility, as determined by Leech, on four specimens contrasts sharply with these claims:

	Water	Acid	Pepsin	Alkali	Trypsin
Claimed.....	Insoluble	Insoluble	Insoluble	Soluble	Soluble
Found solution...	32 (21-47)	37 (32-42)	15 (12-21)	87 (81-95)	84 (78-96)

The specimens of tannoform vary considerably in solubility. All, however, are fairly soluble in water and in dilute acid. The apparently lower solubility in the pepsin acid indicates that the protein of the pepsin has been precipitated; i.e., that there is a slight astringent effect.

The solution in bicarbonate occurs almost immediately at 40 C. The residue that remains undissolved in bicarbonate does not yield formaldehyd on steam distillation, or to 10 per cent. sodium hydroxid. This is evidently an impurity that would be useless therapeutically.

Of the part that goes into solution in bicarbonate, about one-third was still precipitated by acidulation, even after digestion with the bicarbonate at 40 C., for three and one-half hours. This part therefore has not been split into its components, as claimed.

The chemical data indicate that tannoform is not a single substance as the formula would imply, but that it is a mixture of unknown substances of problematical behavior.

Egg albumin Precipitation by Tannoform.—No precipitate occurred in the N/15 acid solution; in the bicarbonate solution, 57 per cent. of the egg nitrogen was precipitated. Tannoform in the acid medium is therefore much less precipitant than the albuminate. In an alkaline medium, it is quite effectively precipitant, although not as active as the albuminates or acetannin.

Serum Precipitation by Tannoform.—The digests were made with 2 or 2.5 per cent. of the tannin preparations. The filtrates of these were diluted with equal volumes of the serum solutions. The results are shown in Table 11

The tannoform is nonprecipitant in neutral digest, or in acid digests. After neutralization, the acid digest is feebly precipitant. The alkaline digests are feebly precipitant, before and after neutralization.

Antihemolytic efficiency of tannoform.—The results are shown in Table 12. "Laking" is equivalent to *absence* of astringency.

TABLE 11.—SERUM PRECIPITATION BY TANNOFORM

Menstruums	2 Per Cent. of Specimen 4	2.5 Per Cent. of Specimen 3
Neutral saline	No precipitate	No precipitate
Hydrochloric acid	No precipitate	
Hydrochloric, then neutralized	Turbid	
Pepsin acid	No precipitate	
Pepsin acid, then neutralized	No precipitate	No precipitate
Bicarbonate, alkaline	Turbid	
Bicarbonate, alkaline, then neutralized	Turbid	
Trypsin, alkaline	Turbid	
Bile, alkaline	Turbid	
Carbonate, then neutralized	Slight precipitate	

One specimen did not protect against laking in neutral, neutralized acid, or alkaline digests. The other specimen conferred partial protection in the neutral digest, and complete protection in the bicarbonate digest.

Kobert found the agglutinative efficiency half as great as with tannic acid. This conclusion seems incredible.

TABLE 12.—TANNOFORM ON HEMOLYSIS

Menstruums	2 Per Cent. of Specimen 4	2.5 Per Cent. of Specimen 3
Neutral saline	Laked	Partly laked
Hydrochloric acid, then neutralized	Laked	
Bicarbonate, alkaline	Laked	No laking
Trypsin, alkaline	Laked	
Carbonate, then neutralized		Laked

Astringent Taste of Tannoform Solutions.—The filtrate of the 2.5 per cent. digest tasted slightly astringent. The neutralized acid pepsin digest was not astringent; the neutralized trypsin bicarbonate digest was doubtful.

Astringent Taste of Tannoform Powder.—All specimens are quite astringent from the start; more so than any of the other masked tannin compounds.

Tannoform on Lung Elasticity.—The extension of the lung is markedly diminished by the saline and still more by the neutralized acid-pepsin digest, thus:

Neutral saline, 2.5 per cent., Specimens C and 3: Marked astringent effect, somewhat more than other masked compounds, and about equal to 0.5 or 1 per cent. tannin. Lungs appear hardened.

Neutralized pepsin acid: Even more astringent than the saline digest.

Neutralized bicarbonate: The extension seems to be but little altered; but this is probably a technical error (leakage?), for the lung appears semi-leathery.

Summary of Tannoform.—This is an impure product, with variable composition. It is somewhat soluble in water and more so at the gastric acidity. Its astringent characters resemble those of albutannin rather than acetannin; i.e., it would act in the stomach more than does acetannin. There are no data as to the liberation of formadlehyd, but free formaldehyd would be therapeutically undesirable.

It is difficult to conceive any advantage of tanniform over the albutannin or acetannin. It is therefore superfluous; and in view of the possible formaldehyd liberation, it appears theoretically undesirable.

TANNOPIN

This is said to be a definite condensation product of tannic acid and hexamethylenamin.

Solubility of Tannopin.—Tannopin is also claimed to be insoluble in water and dilute acids, but to be decomposed by dilute alkalis and then to act as tannin.

Leech's examination of a specimen shows the usual discrepancy between the claims and the findings:

	Water	Acid	Pepsin	Alkali	Trypsin
Claimed.....	Insoluble	Insoluble	Insoluble	Soluble	Soluble
Found solution...	17	32	20	26	24

The claimed contrast of solubility in alkalis as against water and acid does not exist. The solubility characters speak against therapeutic efficiency; although it is conceivable that the action in the lower intestines might be more efficient than with more soluble compounds.

Egg-Albumin Precipitation by Tannopin.—None was precipitated in the acid medium; 50 per cent. was precipitated in the bicarbonate medium. The bicarbonate precipitation is about the same as with tannoform, and smaller than with albutannin or acetannin.

Tannopin on Serum Precipitation and Hemolysis.—The digests were made with 2 per cent. of Specimen 12. The filtrates of these were diluted with equal volumes of the serum solutions. The results are shown in Table 13. With the blood experiments, laking means that the solution is *not astringent.*

Astringent Taste of Tannopin Solutions.—Filtrate of the 2.5 per cent. digests have little or no astringent taste. The neutral saline and the neutralized pepsin hydrochloric digests were nonastringent. The neutralized trypsin-bicarbonate digest was doubtful.

Astringent Taste of the Powder.—Two specimens were entirely nonastringent.

TABLE 13.—TANNOPIN ON SERUM PRECIPITATION AND HEMOLYSIS

Menstruums	Serum	Blood
Neutral saline	No precipitate	One laked, another partly laked
Hydrochloric, acid	No precipitate	
Hydrochloric, neutralized	No precipitate	Doubftul
Pepsin, acid	No precipitate	
Pepsin, neutralized	Turbidity	
Bicarbonate, alkaline	No precipitate	One laked, another not laked
Trypsin, alkaline	No precipitate	Laked
Trypsin, neutralized	Turbidity	
Carbonate, neutralized	Slight precipitate	Not laked

Lung Elasticity.—This was not tested.

Summary of Tannopin.—This is only very feebly astringent; about equally so in neutralized acid and neutralized alkaline digest. It is apparently less effective than albutannin or acetanin, and is at least superfluous.

TANNISMUTH

Composition and Solubility.—Tannismuth is said to be bitannate of Bismuth, approximately $Bi(OH)(C_{14}H_9O_9)_2$. It is claimed as insoluble in water, but soluble in dilute acids. It is also claimed that one molecule of tannin is split off at once on ingestion, but that the other tannin molecule is split off more slowly, so as to act in the intestine. It is employed against diarrhea, to produce "the combined effect of bismuth and tannic acid."

Leech found a specimen to behave as follows :

	Water	Acid	Pepsin	Alkali	Trypsin
Percentage soluble...	18.2	25	14.8	14.6	12.8

These findings indicate that a part dissolves in water and somewhat more in dilute acid. The pepsin experiment shows that this can precipitate protein and is presumably tannin. There is, however, no indication that any further tannin is split off by bicarbonate. In fact, the solubility in alkali is even less than in water. The preparation would act essentially after the manner of insoluble bismuth salts, plus a slight tannin effect that would probably be expended entirely on the stomach.

Tannismuth on Serum Precipitation and Hemolysis.—The digests were made with 2 per cent. of Specimen 10. The filtrates were diluted with equal volumes of serum solution. The results are shown in Table 14. In the blood experiments, laking means that the solution is *not* astringent.

Taste of Tannismuth Powder.—This is distinctly astringent from the start, ranging between tannalbin and protan.

TABLE 14.—TANNISMUTH ON SERUM PRECIPITATION AND HEMOLYSIS

Menstruums	Serum	Blood
Neutral saline	No precipitate	One laked, another not laked
Hydrochloric, neutralized	Slight precipitate	One partly laked, another not laked
Pepsin acid, neutralized	Precipitate	Not laked
Bicarbonate, alkaline		Not laked
Bicarbonate, neutralized	Slight precipitate	Laked
Trypsin, neutralized	Precipitate	Not laked

Summary of Tannismuth.—The tannin of this preparation seems to be equally soluble in acid and in alkaline mediums. Its astringent action would probably be expended in the stomach, so that it would act in the intestines probably mainly as an insoluble bismuth salt.

GALLOGEN

Comparison and Salubility.—"Gallogen" is described as anhydrous ellagic acid. It is claimed to be insoluble in water and dilute acids, and soluble to 2 per cent. in alkaline mediums. It is thus supposed to dissolve slowly in the intestines and to act there as an astringent. The solutions oxidize readily.

The specimen examined by Leech behaved thus:

	Water Insoluble	Acid Insoluble	Pepsin Insoluble	Alkali Sparingly soluble	Trypsin Sparingly soluble
Claimed					
Percentage found soluble	18		15.9	14.9	17.2

The data show that the compound is sparingly and about equally soluble in all the mediums.

Egg-Albumin Precipitation by Gallogen.—None was precipitated in N/15 acid. In bicarbonate, 58 per cent. of the albumin was precipitated.

Gallogen on Serum Precipitation and Hemolysis.—The digests were made with 2 per cent of Specimen 11. The filtrates were diluted with equal volumes of serum solution. The results are shown in Table 15. In the blood experiments, laking means that the solution is *not* astringent.

Taste of the Powder.—This is at first slightly salty and acid, then slightly astringent, ranging between tannigen and tannalbin.

TABLE 15.—GALLOGEN ON SERUM PRECIPITATION AND HEMOLYSIS

Menstruums	Serum	Blood
Neutral saline	No precipitate	One laked, another not laked
Hydrochloric, neutralized	No precipitate	One laked, another not laked
Pepsin acid, neutralized	No precipitate	Laked
Bicarbonate, alkaline		Not laked
Bicarbonate, neutralized	No precipitate	Not laked
Trypsin, neutralized	No precipitate	Laked

Summary of Gallogen.—This is feebly astringent, rather more so with alkaline than with acid digests (after neutralization). The chemical data are not sufficient to define its probable usefulness.

THE INFLUENCE OF PEPSIN IN ACID DIGESTS ON THE ASTRINGENCY OF MASKED TANNINS

It is generally stated that peptic digestion diminishes the precipitation of proteins by tannin. This is probably an error based on neglect

TABLE 16.—COMPARISON OF HCL AND PEPSIN HCL

	Percentage Dissolved in		Serum Precipitation (After Neutralization) By	
	HCl	Pepsin HCl	HCl	Pepsin HCl
Protan	37	79	+	++
Tannalbin	3	76	t slight	+
Tannopin	20	26	0	t
Tannigen	5.8	6.2	t	t
Tannismuth	25	15	+	+
Gallogen	20	16	0	0
Tannoform	37	15	t	0

of the influence of the reaction in digestion experiments; for, as was shown in the first paper of this series, peptones and native proteins behave essentially alike toward tannin.

There is another manner, however, in which pepsin might affect the astringency of masked tannin compounds, and that is by increasing their solubility. This would be expected with the heat-coagulated protein of tannalbin and with the acid-insoluble casein of protan.

The results are shown in Table 16.

THE INFLUENCE OF TRYPSIN IN BICARBONATE DIGESTS ON THE ASTRINGENCY OF MASKED TANNINS

Trypsin might conceivably influence the solubility and therefore the activity of these compounds; in the case of the albuminates, by dissolving coagulated albumin; in the case of esters, by promoting their saponification.

The results of Leech (Table 17) showed that trypsin does actually increase the solubility of tannalbin, but that it has no effect on any of the other masked compounds.

TABLE 17.—INFLUENCE OF TRYPSIN ON SOLUBILITY IN BICARBONATE (FROM LEECH)

	Percentage Dissolved in	
	Bicarbonate	Bicarbonate and Trypsin
Tannalbin	45	60
Protan	90	87
Tannigen	82	76
Tannoform	94	93
Tannopin	24	24
Gallogen	15	17

The precipitation and hemolysis experiments indicate that the trypsin-bicarbonate digests are somewhat less astringent than the simple bicarbonate digests; but this doubtless is deceptive; i.e., precipitation of the trypsin-protein has already bound a part of the tannin.

THE INFLUENCE OF BILE SALTS ON THE ASTRINGENCY OF MASKED TANNIN

Since bile salts occur in the intestines and increase the solubility of some substances, they were tried in some of the precipitation experiments with tannalbin, tannigen and tannoform. The results were negative, i.e., identical with those of bicarbonate in the absence of bile salts.

SUMMARY AND CONCLUSIONS

The astringencies of "masked tannin compounds" were compared with each other, and those of tannic acid, under conditions simulating those occurring in the digestive tract. Various criteria of astringency were applied, including a new method employing blood corpuscles. All methods gave fairly concordant results. The simplest, and therefore the most satisfactory appeared to be the precipitation of protein solutions. This, in relation to the solubility and taste of the drug, should give a fairly complete picture of its field of usefulness.

Great variations in the composition and properties of different specimens of the commercial products render their exact classification difficult. However, the following conclusions appear justified:

Tannin-Protein Compounds.—Contrary to prevailing opinions, these dissolve rather better in artificial acid-gastric juice than in bicarbonate-trypsin solutions. Their solubility is so slow, however, that they could be only silghtly astringent in the stomach. Considerable astringency would develop in the duodenum, when the acidity is reduced; and the effects would continue somewhat into the lower intestine, following the cleavage of the undissolved proteinate. Slow solubility in bicarbonate solution is therefore a desideratum if the action is intended to continue beyond the duodenum.

Tannin-Acetyl Esters.—Commercial brands are evidently mixtures of varying quality; some specimens apparently containing considerable

free tannin. The best specimens, however, appear fairly uniform and but slightly soluble and astringent in acid solution. Bicarbonate dissolves them, and hydrolyzes them slowly. The astringency goes parallel to the hydrolysis, so that the action would continue for several hours, and could thus extend into the lower intestines. However, they do not deserve full confidence until the commercial products are more uniform.

Other Compounds.—The properties of those that were investigated do not appear promising.[3]

3. In addition to the references given, the following will be found of interest:
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SOME FACTORS RELATING TO THE TOXIC ACTION OF ARSPHENAMIN*

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There is little unanimity of opinion as to the cause of the untoward effects that not infrequently follow the administration of arsphenamin. Some apparently believe that, with the present quality of arsphenamin on the American market, untoward reactions must be ascribed to carelessness on the part of the physician; but just how this carelessness operates—what physical or chemical changes are brought about in the drug, for example, or what physiologic effects are supposed to be caused by the use of too concentrated solutions or by too rapid injection—is not stated. On the other hand, some clinicians think that the quality of the drug is almost always at fault when untoward results are obtained.[1]

Without entering on a discussion of this question, the fact remains that there have been wide variations in the toxicity of preparations of arsphenamin not only as they are prepared by standard methods in different laboratories, but also among those on the market, and that the average of the commercial products is below that of preparations which may readily be made in the experimental laboratory. It is the purpose of the work (of which this is a preliminary report) in progress in this laboratory to investigate the causes of this difference and also the changes, chemical or physical, by which a "good" preparation may lead to untoward results.

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* The experiments described in this report were begun under the auspices of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association, and are being continued under a grant from the United States Interdepartmental Social Hygiene Board.

1. Compare, for example, Pusey, W. A.: Administration of Arsphenamin, J. A. M. A. **72**: 1786 (June 14) 1919.

TYPES OF TOXIC ACTION

It may be stated at once that in experimental studies, both with commercial preparations and with preparations prepared by Mr. Christiansen in this laboratory, three distinct types of toxic action have been observed: (*a*) preparations the toxicity of which was due to the presence of m-amino-p-hydroxyphenylarsenous oxid ("arsenoxid"); (*b*) preparations the toxicity of which seemed to be due to the presence in the manufactured product of toxic substances other than the above mentioned oxid, and (*c*) preparations toxic on account of the physical properties of the solution or of the presence in them of a very easily destroyed toxic substance.

The third of the foregoing classes of preparations will be chiefly considered in this paper, but a few words may be said in regard to the other two.

TOXICITY DUE TO THE PRESENCE OF "ARSENOXID"

Ehrlich attributed the toxic action of arsphenamin largely to the formation of m-amino-p-hydroxyphenylarsenous oxid ("arsenoxid"), and this has been the most frequently discussed possibility. The possibility of this oxid's being involved in some of the clinical reactions of arsphenamin has recently again been raised by Smith [2] on the basis of a pharmacologic study of its action. A number of workers, however, have shown that there is a lack of parallelism between the amount of this oxid present in samples of arsphenamin and the toxic action of the latter, so that it cannot be the sole cause of untoward reactions.

I have not encountered a toxic commercial preparation of arsphenamin or an experimental preparation (except when special care was taken during the manufacture to secure such) the toxicity of which could be attributed to the oxid. But the evidence at present available suggests that the chief, if not the only, change leading to increased toxicity occurring in solutions of arsphenamin as the result of standing, shaking, or warming in the presence of air is due to the formation of this oxid or an analogous substance. Ehrlich [3] reported that the toxicity of a solution of arsphenamin

2. Smith, M. I.: J. Pharmacol. & Exper. Therap. **15**: 279 (June) 1920.
3. Ehrlich, P.: Abhandlungen über Salvarsan **3**, 1913.

became one and a half times greater when the solution was allowed to stand exposed to the air for four hours.

Roth [4] is apparently the only one who has published figures as to the increased toxicity of arsphenamin when alkaline solutions are shaken in the presence of air; he found that the toxicity of the preparation with which he worked increased 60 per cent. after one minute's vigorous shaking. Unfortunately, Roth reports results with only a single preparation. I have aerated alkaline solutions by drawing a slow current of air (first passed through sodium hydroxid to remove the carbon dioxid) through them at room temperature and found great variations in the effects on different preparations. With some preparations there was a marked increase in the toxicity within an hour; with others there was little change until after four hours' aeration, although the rate at which the air was drawn through was approximately the same. Voegtlin and Smith [5] have followed the oxidation of alkaline solutions of arsphenamin quantitatively without, however, recording the changes in toxicity.

In toxicologic experiments it has been assumed rather than actually demonstrated that the increased toxicity was due to oxidation. My experiments offer a certain amount of proof that the oxid, or at least a partially oxidized but at the same time easily reducible substance, is the chief factor.

In the first place, the symptoms produced by a specimen of arsphenamin which has been aerated or shaken are those characteristic of the oxid and are quite unlike those caused by other, unknown, toxic factors which will be discussed in the next section. These symptoms consist (in rats) in struggling, convulsive movements, lashing of the tail, rigidity of the legs, irregular respiration with long pauses, protrusion of the eyes, lacrimation and salivation; they begin promptly, often before the injection is completed. Roth has aptly described these symptoms as a "resistance to the injection." I have endeavored to determine how small an amount of oxid either alone or mixed with arsphenamin could, with a high degree of probability, be detected

4. Roth, G. B.: Pub. Health Rep. **35**: 2205 (Sept. 17) 1920; Toxicity of Arsphenamin and Neo-Arsphenamin, Arch. Dermat. & Syph. **2**: 292 (Sept.) 1920.

5. Voegtlin, C., and Smith, H. W.: J. Pharmacol. & Exper. Therap. **16**: 199, 1920.

by the symptoms; the symptoms were distinct with 5 mg. per kilogram of rat (about one fifth the fatal dose) and very pronounced with 10 mg. per kilogram. When 10 per cent. of arsphenamin in a sample was replaced by an equal weight of the oxid and injected into rats in doses of from 100 to 120 mg. per kilogram, the symptoms were characteristic of the oxid, and entirely unlike those caused by any other compounds I have tested, with the exception of the analogous sulphid.

Another indication that the oxid is the toxic agent in samples of arsphenamin which have been agitated with air is the fact that such solutions may be rendered relatively nontoxic by the means that reduce the toxicity of the oxid. Thus, if sodium hydrosulphite is added to a solution of the "oxid," the toxicity is immediately reduced; I have not determined the limit of this detoxication, but have found that more than double the fatal dose of the oxid could be rendered nontoxic by this means. If a solution of arsphenamin is rendered toxic by aeration, the typical "oxid" symptoms and the increased toxicity can be at once overcome by sodium hydrosulphite. Thus, a preparation of arsphenamin which did not kill in doses of 100 mg. per kilogram was aerated by drawing air through it at room temperature until it produced typical oxid symptoms and killed in a dose of 60 mg. per kilogram; sodium hydrosulphite was added; now doses of 100 mg. caused neither symptoms nor death.

A solution of arsphenamin made toxic by aeration at room temperature can also be rendered less toxic by continued aeration with warming. Thus, part of the toxic solution used in the foregoing experiment was aerated for two hours at a temperature varying from 45 to 60 C.; the injection of what corresponded to 100 mg. per kilogram caused no immediate symptoms; and although later tremors and a tendency to circus movements developed, the rats ultimately completely recovered; as noted above, this solution before the second aeration produced immediate "oxid" symptoms and caused death in doses corresponding to 60 mg. per kilogram. The toxicity of a solution of m-amino-p-hydroxyphenylarsenous oxid could be lowered in a similar manner by aeration. Thus, an alkaline solution of the oxid was aerated at about 50 C. for an hour and a

half; doses corresponding to 80 mg. per kilogram of the oxid caused no symptoms whatever and did not cause death, although 25 mg. before the aeration caused immediate typical symptoms followed by death.

The loss of toxicity of aerated arsphenamin and of the "arsenoxid" is doubtless due to the further oxidation, with formation of m-amino-p-hydroxyphenylarsonic acid, which is much less toxic than arsphenamin.

A solution of arsphenamin aerated at 56 C. for an hour became slightly less toxic and did not cause oxid symptoms; a portion of the same solution aerated at room temperature for the same period became much more toxic and caused oxid symptoms. In the former case the oxid was apparently further oxidized as it was formed.

It did not seem possible to increase the toxicity of arsphenamin to a point corresponding to its complete conversion into the oxid. The most toxic preparations that I obtained required an amount of the solution corresponding to nearly 60 mg. of arsphenamin per kilogram; this is about the degree of toxicity obtained by Roth in his shaking experiment.

A solution of arsphenamin made toxic by aeration differs from the solutions of at least some toxic preparations of arsphenamin in that when the arsphenamin base is precipitated by neutralization (to litmus) and the solution filtered, the filtrate is toxic and causes the typical "oxid" symptoms. The filtrate may be rendered nontoxic in the same way as the original solution or a solution of the pure oxid itself, that is, by treatment with hydrosulphite or by aeration.

The foregoing methods afford a ready means of determining whether the toxicity of a solution of arsphenamin is due to the "arsenoxid" (or some very similar substance).

When the (acid) solution of arsphenamin was aerated for from two to four hours, there was no increase in the toxicity; Voegtlin and Smith showed that there was little oxidation of arsphenamin under such conditions.

Only two, incidental, experiments were made on the effect of heating the acid solution; in these the dihydrochlorid was dissolved in very hot water and kept in an almost boiling water bath for an hour. In both cases

there was a distinct but not marked increase in toxicity; the toxicity was lowered by treatment with sodium hydrosulphite.

TOXIC SUBSTANCES OTHER THAN "ARSENOXID" WHICH MAY BE PRESENT IN ARSPHENAMIN

There were formerly on the market preparations of arsphenamin much more toxic than those found at present; such products are not infrequently obtained in the process of manufacture and prevented from coming into use by the present higher standards of the U. S. Public Health Service [6] and, as has already been stated, the average toxicity of the commercial products in use is greater than that of products that may readily be obtained in the experimental laboratory.[7] This greater toxicity cannot be attributed to the presence of "arsenoxid," and most writers have taken for granted the presence in such samples of small amounts of substances much more toxic than arsphenamin itself. This view has not, however, been universally accepted. Thus, the British Salvarsan Committee [8] states: "There is no evidence, indeed, to justify the conclusion that ordinary variations of toxicity, apart from those due to exposure of the substance to air, are due to the presence of impurities more toxic than 606 itself," and then the committee suggests a physical theory of the toxic action of the drug (which will be discussed later). The products considered by this committee to be "good" had, however, a higher toxicity than the preparations now permitted to be sold in this country.

Some of the American investigators have from the first maintained that there is present, in very small amounts, in some samples of arsphenamin a substance or substances much more toxic than arsphenamin

6. The U. S. Public Health Service has gradually raised the requirements as to the tolerated dose for rats from 50 to 100 mg. per kilogram.

7. Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W. (Am. J. M. Sc. **160**: 188 [Aug.] 1920) found the average tolerated doses of the products of six laboratories to be between 100 and 110 mg. per kilogram of rat, whereas the average tolerated doses of the preparations made in this laboratory by Mr. Christiansen by the method recently described by him (J. Am. Chem. Soc. **42**: 2402, 1920) was between 140 and 150 mg. per kilogram.

8. Reports of the Special Committee upon the Manufacture, Biological Testing and Clinical Administration of Salvarsan and of Its Substitutes, No. 1, Medical Research Committee, Special Report Series, No. 44, 1919.

itself.[9] All attempts to identify such substances by various refinements of analysis[10] (determination of As: N ratio, etc.) have failed. We have not succeeded in identifying a toxic substance or substances, but have been able to exclude some of the possibilities that have been suggested, and have learned something as to the conditions under which toxic products result. Inorganic arsenic compounds have been suggested as the toxic agent; although traces of these have occasionally been found in toxic preparations of arsphenamin which we have tested, the amounts have been far too small to account for the abnormal toxicity. It has also been suggested that the toxicity may be due to sulphur compounds. Arsphenamin made, as is usually the case, by the use of sodium hydrosulphite as the reducing agent, always contains small but variable amounts of sulphur. Ehrlich and Bertheim[11] stated that in the process of reduction, compounds in which sulphur is attached to the arsenic atom are liable to be formed and that these are highly toxic; no details are given as to the nature of these compounds or the degree of their toxicity. The British committee found no correspondence between sulphur content and toxic action. Mr. Christiansen prepared the 3-amino-4-hydroxyphenylarsenous sulphid; the fatal dose of this (intravenous injection into rats) was between 40 and 50 mg. per kilogram. When 6.6 per cent. of a preparation of arsphenamin, which had a tolerated dose of 110, was replaced by this sulphid, the tolerated dose of the mixture was 100 or a little less. Thus, while this sulphid is more toxic than arsphenamin, the presence of even a relatively high percentage would not account for the abnormal toxicity of the class of preparations under discussion.

Furthermore, the symptoms caused by this sulphid are very severe and are very similar to those caused by the corresponding oxid; thus, the mixture containing 6.6 per cent. of the sulphid caused violent symptoms unlike those following any other preparations I have tested with the exception of those containing the

9. Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W.: J. Cutan. Dis. **35**: 286 (May-June) 1917.

10. Raiziss, G. W., and Proskouriakoff, A.: The Chemistry of Arsphenamin and Its Relation to Toxicity, Arch. Dermat. & Syph. **2**: 280 (Sept.) 1920.

11. Ehrlich, P., and Bertheim, A.: Ber. d. deutsch. chem. Gesellsch. **44**: 1260, 1911.

"oxid." Thus, the presence of an amount of this sulphid too small to increase greatly the toxicity of a preparation of arsphenamin is easily detected by the symptoms.

The question of whether there are other toxic sulphur compounds which can account for the toxicity of some preparations of arsphenamin is being investigated.

Among other possible contaminations is a polyarsenid of the formula $(C_6H_3NH_2OH)_2\ As_4$,[12] which has been stated to be more toxic than arsphenamin itself; I have seen no figures to support the last statement, and my own experiments show that rats tolerate doses of 140 mg. per kilogram (intravenous injection); that is, this substance is distinctly less toxic for rats than average samples of arsphenamin.

These toxic arsphenamin preparations differ from those the abnormal toxicity of which is due to the arsenoxid in that the symptoms are different; when the greater part of the arsphenamin is precipitated from the solutions by neutralization the filtrate is not toxic, and the toxicity of the solutions is not lessened by treatment with sodium hydrosulphite. When the solutions were aerated, marked "oxid" symptoms developed, although there was but little increase in toxicity. Warming the solutions for a short time did not diminish the toxicity, as was the case with the class of toxic preparations discussed in the next section.

Until a toxic compound can be isolated and identified it cannot be stated that the toxicity of the class or classes of preparations under consideration is really due to the presence of substances more toxic than arsphenamin itself; but the probability that such is the case is very great.

IS THE TOXICITY OF A SOLUTION OF ARSPHENAMIN INFLUENCED BY ITS PHYSICAL STATE?

Danysz,[13] as a result of experiments which for the most part have not been accepted,[14] attributed many of the reactions of arsphenamin to the precipitation of the latter by certain constituents of the blood. A some-

12. Christiansen, W. G.: J. Am. Chem. Soc. **42**: 2402, 1920; **43**: 370, 1921.
13. Danysz, J.: Ann. de l'Inst. Pasteur **17**: 114, 1917.
14. Schamberg, J. F.; Kolmer, J. A.; Raiziss, G. W., and Weiss, C.: Laboratory and Clinical Studies Bearing on Causes of Reactions Following Intravenous Injections of Arsphenamin and Neo-Arsphenamin, Arch. Dermat. & Syph. **1**: 235 (March) 1920.

what similar view was expressed by the Salvarsan Committee of the British Medical Research Committee [15] in these words:

> There is a good deal of evidence in support of the view that the varying toxicity of different samples of "606" is related to small differences in the physical properties of the product, which determine the state of physical dispersion in which the free base comes out of solution when the alkaline solution is injected intravenously.

What the evidence for this view is, is not very fully stated. An instance is cited, however, in which samples of arsphenamin dissolved in tap-water caused symptoms of vasomotor disturbances whereas, when dissolved in distilled water, no symptoms resulted, while other samples could be dissolved in tap-water with impunity. The authors conclude:

> It would be very difficult to reconcile such an experience with the view that abnormal toxicity depends on the presence of an undue proportion of more toxic by-products.

Recently in working with certain lots of arsphenamin I have observed phenomena which strongly support the idea that the physical condition (colloidal?) of the solution or the presence of a very labile toxic compound may at times account for untoward reactions.

Physicians are usually warned, when making solutions of arsphenamin, not to warm them, since, it is stated, the toxicity is thereby greatly increased. In endeavoring to determine to what extent the toxicity of a solution is increased by different degrees of warming, it was found that in many cases there was not only no increase but a marked decrease in toxicity. Experiments 1, 2 and 3 are typical:

> EXPERIMENT. 1 (R^3 37).—A 2 per cent. solution of arsphenamin was made alkaline at 2:05 p. m. and divided into three parts; one was kept at room temperature, another at 43 C. and the third at 60 C. Intravenous injections into rats were made as recorded in Table 1.
>
> EXPERIMENT 2 (R^3 33).—A solution of arsphenamin was made alkaline, and one half was warmed to slightly above 40 C. for a few minutes, and then allowed to come to room temperature; the other half remained at room temperature (Table 2).

15. Reports of the Special Committee upon the Manufacture, Biological Testing and Clinical Administration of Salvarsan and of Its Substitutes. No. 1. London, 1919.

TABLE 1.—RESULTS OF INJECTIONS IN EXPERIMENT 1*

Time, P. M.	Solution at Room Temperature	Solution at 43 C.	Solution at 60 C.
2: 39	80 mg. per kilogram; † 35 min.		
2: 45	.....	.....	80 mg. per kilogram; 0
2: 56	.....	80 mg. per kilogram; 0	
3: 01	.....	.....	80 mg. per kilogram; 0
3: 09	80 mg. per kilogram; † 1 hour		
3: 23	.....	100 mg. per kilogram; 0	
3: 34	80 mg. per kilogram; † 12 min.		
3: 42	.....	.....	100 mg. per kilogram; 0

* In the tables, 0 indicates that the rat recovered, and † that the rat died.

TABLE 2.—RESULTS OF INJECTIONS IN EXPERIMENT 2

Time, A. M.	Solution at Room Temperature	Warmed Solution
11: 34	80 mg. per kilogram; † 8 min.	
11: 38	80 mg. per kilogram; † 2½ hours	
11: 50	.....	100 mg. per kilogram; 0 (sick)
12: 05	.....	80 mg. per kilogram; 0
12: 08	80 mg. per kilogram; † 6-15 hours	
2: 32	.....	80 mg. per kilogram; 0
2: 36	80 mg. per kilogram; † 25 min.	
3: 18	80 mg. per kilogram; † 3-15 hours	
3: 22	.....	80 mg. per kilogram; 0
	Ice box 42 hours	
9: 46	80 mg. per kilogram; 0 (very sick)	
9: 51	.....	80 mg. per kilogram; 0
10: 30	.....	100 mg. per kilogram; † 55-65 hours
10: 35	80 mg. per kilogram; † 8 days (very sick)	
P. M.		
12: 23	100 mg. per kilogram; † 10-25 min.	
12: 23	.....	100 mg. per kilogram; 0
4: 54	.....	120 mg. per kilogram; † ¼-25 hours
4: 59	.....	120 mg. per kilogram; 0

Thus, warming at 40 C. for a few minutes rendered the solution permanently less toxic. The part of the solution which was not warmed retained its undue toxicity for at least forty-eight hours.

EXPERIMENT 3 (R^3 41).—A solution of arsphenamin was found to be toxic; a part of it was removed and warmed to 55 C. for fifteen minutes, while the remainder was kept at room temperature (Table 3).

Two and a half months later another ampule of this preparation, which had been kept, sealed, at the laboratory temperature, was tested. It caused death at 80 mg. per kilogram; the solution was warmed to 60 C. for fifteen minutes; it caused death at 100 mg. per kilogram (smaller doses were not given). When warmed

TABLE 3.—RESULTS OF INJECTIONS IN EXPERIMENT 3

Time, P. M.	Solution at Room Temperature	Warmed Solution
12:10	80 mg. per kilogram; 0	
12:19	80 mg. per kilogram; † 15-20 minutes	
1:00	80 mg. per kilogram; † 2-9 minutes	
1:50	.....	100 mg. per kilogram; 0
2:26	80 mg. per kilogram; † 10-15 minutes	
2:40	.....	120 mg. per kilogram; 0
2:50	.....	100 mg. per kilogram; 0
3:17	.....	120 mg. per kilogram; † 1½ hours
5:11	.....	110 mg. per kilogram; 0

to 60 C. for one and a half hours it did not cause death in doses up to and including 120 mg. per kilogram (it was not tested at higher doses). This experiment shows that this kind of toxicity is preserved for long periods and also suggests that warming has less effect in diminishing the toxicity after the storage. The original test was made within a day after the preparation was made.

Since warming was found to diminish the toxicity of these preparations, experiments were next made to determine if cold would increase or preserve the toxicity. Accordingly, solutions were kept in ice and injected cold; control experiments were made in which

solutions of nontoxic preparations were injected at the same temperature, and it was found that the temperature was not responsible for the differences.

TABLE 4.—RESULTS OF INJECTIONS IN EXPERIMENT 4

Time, P. M.	Solution at Room Temperature	Cold Solution
2: 08	80 mg. per kilogram; 0	
2: 17	110 mg. per kilogram; 0	
3: 33	.....	80 mg. per kilogram; † at once
3: 50	.....	80 mg. per kilogram; † 4-5 minutes
4: 44	.....	60 mg. per kilogram; † 6 minutes

TABLE 5.—RESULTS OF INJECTIONS IN EXPERIMENT 5

Time	Warmed Solution	Cold Solution
1: 40 p. m.	120 mg. per kilogram; 0	
1: 51 p. m.	.....	100 mg. per kilogram; † 1-2 minutes
1: 56 p. m.	.....	60 mg. per kilogram; very sick; recovered
2: 09 p. m.	.....	100 mg. per kilogram; † 10 minutes
2: 14 p. m.	130 mg. per kilogram; 0	
	Cylinder in ice	
2: 28 p. m.	120 mg. per kilogram; 0	
2: 49 p. m.	.....	100 mg. per kilogram; 0
2: 55 p. m.	140 mg. per kilogram; 0	
3: 46 p. m.	.....	120 mg. per kilogram; 0
3: 51 p. m.	150 mg. per kilogram; 0	
4: 36 p. m.	.....	130 mg. per kilogram; 0
	Solution warmed to 40 C.	
4: 40 p. m.	140 mg. per kilogram; 0	
	Cylinder in ice over night; injected cold	
9: 33 a. m.	130 mg. per kilogram; 0	
9: 44 a. m.	150 mg. per kilogram; 0	
12: 29 p. m.	150 mg. per kilogram; 0	

Experiments 4, 5, 6 and 7 are typical of this class:

EXPERIMENT 4 (IV 5).—The acid aqueous solution of a preparation was divided into two portions; one was cooled in ice and then the alkali added; the other portion remained at room temperature and the alkali was added (Table 4).

Immediately after the first injection of the cold solution a portion was removed and warmed to 38 C. for a few minutes

and injected: 100 mg. per kilogram did not cause death. This solution was then placed in ice and when thoroughly cold was injected, still cold, in doses up to and including 110 mg. per kilogram without causing a single death.

EXPERIMENT 5 (IV 11).—The contents of an ampule were divided into two portions; one portion was dissolved in ice-cold distilled water, the other in distilled water at 41 C. Sodium hydroxid was added to each, and the solutions were kept at these temperatures and injected (Table 5).

Thus, the solution of this preparation which had been warmed to 41 C. did not kill a single rat in doses up to and including 150 mg. per kilogram; this was true whether the solution was injected at near the body temperature or near zero.

TABLE 6.—RESULTS OF INJECTIONS IN EXPERIMENT 6

2:03	100 mg. per kilogram; 0
2:20	120 mg. per kilogram; 0

TABLE 7.—RESULTS OF INJECTIONS IN EXPERIMENT 7

Time	
11:45 a. m.	100 mg. per kilogram; † 2 minutes
11:52 a. m.	80 mg. per kilogram; † 4-5 minutes
11:57 a. m.	60 mg. per kilogram; † 13 minutes
12:02 p. m.	80 mg. per kilogram; † 30-60 minutes
12:06 p. m.	100 mg. per kilogram; † 2 minutes
1:09 p. m.	80 mg. per kilogram; 0
1:50 p. m.	100 mg. per kilogram; 0

This experiment also shows that there was a diminution in the toxicity of a solution although it was kept cold.

That this diminution is hastened by warming is shown by Experiment 6.

EXPERIMENT 6.—At 1:58 p. m. (at a time when it was fatal in a dose of 100 mg. per kilogram), part of the cold solution was removed and warmed to 38 C. and injected (Table 6).

In this experiment the toxicity of the solution diminished on standing, although it was kept cold. This was observed in other experiments also. Thus:

EXPERIMENT 7.—A preparation (IV 1) was dissolved in water, and the cylinder containing the solution was placed in ice; when the solution was cold the alkali was added and injections made (Table 7).

The solution was then warmed to 40 C. for a few minutes, cooled to room temperature and injected; rats now survived doses of 130 and 140 mg. per kilogram.

Attention may be called to the fact that in the case of the injections of the cold solution in this experiment the solution was kept in ice between the injections and that when 100 mg. per kilogram was injected 67 per cent. more of the cold solution was injected than when 60 mg. per kilogram was injected; yet the latter caused death, whereas the former, after the solution had stood for two hours, did not. This is another illustration of the fact that the temperature is not the factor causing the greater toxicity of the cold solutions. In fact, rats seem very resistant to the injection of cold solutions—at least when the injection is made at the standard rate used in these experiments, namely, 0.5 c.c. per minute. Mice seemed much more sensitive to the intravenous injections of cold solutions.

Observations were next made of the effect on toxicity of allowing the solutions to stand (in glass-stoppered cylinders) at room temperature; it was found that in some cases there was a diminution of toxicity.[16] There were great differences in this respect with different preparations. In Experiment 2 a preparation, the toxicity of a portion of which was at once diminished by warming to 40 C. for a few minutes, remained toxic for nearly four hours when left at room temperature; and even after standing in the icebox for forty-two hours it was more toxic than a portion of the same solution which had received the same treatment except for the few minutes' warming at 40 C. With other preparations the toxicity diminishes rapidly and the results are like those noticed by the observers quoted. For example, with one preparation (77 b-1) a dose of 110 mg. per kilogram, injected immediately after being made alkaline, caused death, as did also a dose of 130 mg. per kilogram injected twenty-three minutes later; thereafter, in the course of four hours, a number of injections of doses up to and including 150 mg. per kilogram were made without there being a death. The solution was kept at room temperature between the injections. The effect on toxicity of standing (and of warming a solution) was also shown by such experiments as these: one half of a sample (V_1) was dissolved in water at room temperature and made alkaline;

16. This observation is not new, although so far as I am aware nothing has been published on it. Dr. G. C. Lake, some time ago, remarked that he had noticed that the first one or two rats injected with a given dose of arsphenamin frequently died, whereas those injected later survived; Drs. G. W. McCoy and G. B. Roth stated that the records of the Hygienic Laboratory showed the same thing. My own records of the toxicity tests made on the arsphenamin of the Massachusetts Department of Health did not show such a phenomenon except rarely.

the first injection (100 mg. per kilogram) caused death; later injections made at ten-minute intervals did not kill in doses of 120, 130 and 140 mg. The other half of the sample was dissolved in water at 40 C., alkali added and the solution injected at once; the first injection (100 mg. per kilogram) was not followed by death as was the case when the sample dissolved and neutralized at room temperature was injected. Many other illustrations of this phenomenon could be cited.

Whether or not warming the acid solution will diminish the toxicity of this class of preparations has not been definitely determined.

The toxicity which is diminished by warming the alkaline solution is evidently of a different type from those discussed in the first two sections of this paper.

The warming of solutions, the toxicity of which is due to the presence of "arsenoxid," does not diminish the toxicity unless the warming is continued for some time and the solution is aerated (oxidation of the oxid), nor does the warming have any effect on the toxicity of preparations discussed in the second division of this paper.

The cause of this type of toxicity has not been determined, but is being made the subject of further study. The symptoms are very suggestive of those of anaphylactic shock, and it seems probable that an analogous condition is brought about probably by some unusual physical condition of the solution. Treatment, however, in the cold, of rat serum with these toxic solutions did not render the serum toxic, nor did the serum have any effect on the toxicity of the solution. A few experiments were made with a small number of inorganic salts to determine whether they would either diminish the toxicity or prolong it; the results were negative.

There is another, but perhaps less probable, possibility which may be considered; that some compound is present which is easily hydrolyzed by alkali, especially when warmed; this would be analogous to the case of acetyl-cholin or aconitin, the solutions of which when warmed undergo hydrolysis with rapid loss of physiologic activity.

As yet no connection has been found between the process of manufacture and the formation of this type of toxic products; they have resulted from the usual hydrosulphite reduction of m-nitro-p-hydroxyphenyl-

arsonic acid, with the conversion of the base into the hydrochlorid by the methyl alcohol-ether method, and also from the reduction of the m-amino-p-hydroxyphenylarsonic acid [17] and m-amino-p-hydroxyphenylarsenous oxid [18] by hypophosphorous acid (with conversion into the hydrochlorid by precipitation with hydrochloric acid).

That products of this type are found in ordinary commercial preparations is indicated by the observations of Lake, McCoy and Roth noted above.

It is evident that this subject may have considerable importance, both from the standpoint of clinical medicine and from that of laboratory control. Thus, if the testing pharmacologist had such a preparation and added the alkali to a warmed solution or did not inject a solution, made at room temperature, at once after the addition of the alkali or did not make the required number of injections in rapid succession, he would probably overlook the toxic character of the product. If the clinician, on the one hand, had the same preparation and (as he is advised to do) injected the solution immediately after adding the alkali or avoided warming the alkaline solution, there would probably be a severe reaction on the part of the patient. If, however, the injection was delayed a little or if the solution was warm when the alkali was added, there probably would be no reaction. That something of this kind may actually occur is suggested by an incident in which there had been a few complaints by physicians of reactions following the use of certain lots of arsphenamin (some ampules of which showed this type of toxicity in varying degree); but another physician who had used the same lots reported that he had never had a reaction from them. On careful inquiry it was learned that this physician had dissolved the drug in hot water and added the alkali to the solution while it was still warm, ("probably at least 50 C."), the method by which the toxicity of this class of preparations is diminished, but also the method by which the toxicity of the drug is generally supposed to be increased.

This phenomenon may account for some of the cases in which there is apparently a lack of correspondence between the reports of clinicians as to untoward reac-

17. Christiansen, W. G.: (Footnote 12, first reference).
18. Christiansen, W. G.: (Footnote 12, second reference).

tions from certain lots of arsphenamin and the laboratory findings as to toxicity; I recall one case in particular in which several clinicions independently and almost simultaneously reported severe reactions from a commercial lot of arsphenamin which, when tested on rats in the laboratory, tested far above the Public Health Service requirements and 50 per cent. higher than another lot which gave rise to no complaints. In this case the manufacturers attributed the reactions to the use by the physicians of too little or too much alkali or to other errors in technic—a rather improbable explanation under the circumstances.

The phenomenon is of interest in another connection: "Idiosyncrasy" has long been supposed to play a rôle in the reaction of some patients to arsphenamin. Thus, it has recently been stated [10] that "undoubtedly, when a number of patients receive arsphenamin of the same lot by the same method of administration and only one or two show reactions, it is safe to assume that they are due to an idiosyncrasy." Obviously, if the one or two showing the reactions were the first to receive the drug, the reactions may have been due to the physical condition of the solution, which spontaneously changed before the injections that were borne without reactions were made.

SUMMARY

The symptoms (in rats) caused by m-amino-p-hydroxyphenylarsenous oxid ("arsenoxid") are very characteristic; the presence in arsphenamin of a percentage of the "oxid" too small to increase greatly the toxicity can readily be detected by the symptoms; confirmatory evidence of the presence of the "oxid" (or of some oxidation product of arsphenamin) in a toxic preparation of arsphenamin is obtained by treating the solution with sodium hydrosulphite: this leads to an immediate diminution of the toxicity if the latter is due to the presence of the "oxid."

No toxic commercial preparations of arsphenamin have been encountered, the toxicity of which could be attributed to the presence of the "oxid."

Some preparations of arsphenamin are very toxic when the solutions are prepared at ordinary room temperature; the toxicity is greatly reduced by gently warming and in some cases by allowing the solutions to stand for a time at room temperature. Cold may

preserve the toxicity for long periods. (Preparations which, after warming, may be tolerated in doses of 150 mg. per kilogram may kill in doses of 60 mg. before warming.) It is considered probable that the undue toxicity of such preparations is due to the physical state of the solution, and that this undergoes a rapid change when the solution is warmed.

Toxic preparations of arsphenamin are sometimes encountered, the toxicity of which is due to factors other than the foregoing: the toxicity is not diminished by the action of sodium hydrosulphite or by warming; it is not due to inorganic arsenic and certain other compounds which have been suggested.

I am indebted to Mr. W. G. Christiansen of this laboratory for the preparation of many of the samples and compounds used in these experiments.

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EVIDENCE FOR THE PRESENCE IN DIGITALIS OF A PRINCIPLE THAT IS ELIMINATED RAPIDLY AFTER INTRAVENOUS INJECTION INTO THE CAT *

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The duration of the cardiac action following the intravenous injection of various digitalis bodies into the cat varies from a period of a day or more after the administration of a nearly fatal dose of strophanthin or of ouabain, to as much as three weeks following the injection of corresponding doses of digtails or digitoxin,[1] but, so far as I know, the literature affords no evidence that any digitalis body exerts a more fleeting action on the cat's heart or that of man than does strophanthin or ouabain.

Hatcher has recently observed (verbal communication) that the cat requires only about half as much of a certain chloroform-soluble fraction of digitalis principles to cause death when it is injected intravenously within a period of a few minutes as it does when the administration is made over a period of four or five hours. This observation is the more surprising in view of the fact that the chloroform-soluble fraction under consideration gives certain of the chemical and biologic reactions characteristic of digitoxin, which behaves in a manner directly opposite to that of this chloroform-soluble fraction with reference to the amounts required to cause death by rapid and by slow administration, respectively.

Hatcher concluded, therefore, that the chloroform-soluble fraction with which he worked consists of a mixture of digitalis principles, of which at least one is eliminated far more rapidly after the intravenous administration in the cat than are other digitalis principles with which we are acquainted.

It is convenient to speak of the "elimination" of an alkaloidal principle when it has ceased to exert its typical effects, even though we may have no definite proof of its destruction, or excretion from the

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* From the Laboratory of Pharmacology of Cornell University Medical College.

1. Hatcher, R. A.: Arch. Int. Med. **10**:268, 1912.

body, and it is in this sense that the term "elimination" is employed in this paper. The body fluids may dilute an irritant substance or they may neutralize an acid, and the animal may recover from the effects of such poisons before they are excreted, but we are accustomed to consider recovery from the effects induced by the administration of a toxic alkaloidal poison as evidence of its destruction, or excretion from the body, and if the administration of an amount of such a poison equal to twice the single fatal dose during a period of several hours or several days is survived, one is justified in supposing that within that period an amount equal to at least a single fatal dose has been eliminated from the body, or rendered incapable of exerting its typical toxic actions. This is especially true when the poison acts directly on the heart, causing it to come to a standstill.[2]

Naturally, this presupposes the absence of any protective mechanism, such as we commonly ascribe to habituation or to the development of tolerance, but such protection is not known to occur with any of the digitalis bodies. Reduction of the activity of the poison by means of the injection of large amounts of fluid, such as Meltzer [3] observed with strychnin, does not come into consideration here; for it is immaterial, so far as the activity of the digitalis bodies is concerned, whether they are administered in fairly dilute, or much more concentrated, solutions; and furthermore, the total amounts of liquids used in most of the experiments did not vary greatly.

Since chemical changes in plant principles are so prone to occur during the process of extraction and purification, it appeared desirable to determine whether the digitalis leaves contain any active principle, such as that which Hatcher supposed to be present in his chloroform-soluble fractions and to be rapidly eliminated after intravenous injection in the cat, and, if so, what percentage of the total activity of the leaf it represents, and also, whether the percentage varies in different specimens of the leaf. The importance of this is obvious in view of the

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2. It is well known that poisons which act on several dissimilar structures exert their several actions during varying periods of time, and after a single toxic dose one frequently sees symptoms referable to one structure long after another structure has recovered completely from the effects of the poison. We must suppose, therefore, that such poisons are retained more tenaciously in those organs in which the action (not necessarily the effects, persist than in those which recover more promptly, and since we have no way of knowing when the last traces of poisons are excreted or destroyed, the term "elimination" is of necessity given some elasticity. Hatcher and Eggleston (J. Pharm. and Exp. Therap. **12**:427, 1919) refer to the elimination of a poison from the organ on which its chief, or characteristic, action is exerted, as evidenced by the return of that structure to its normal behavior toward the poison, that is, its complete recovery, as the "essential elimination."

3. Meltzer: J. Exper. M. **5**:643, 1900-1901.

frequent use of the cat for the biologic standardization of digitalis, and the probability that the elimination in man is more or less similar to that in the cat.

It was supposed that it would be a comparatively simple matter to determine whether such a rapidly eliminated digitalis principle exists in the leaf, in view of the relative constancy with which Hatcher obtained evidences of its pressure in his chloroform-soluble fractions, and the fair degree of uniformity with which cats behave toward a given digitalis body with a given rate of injection; but the results proved somewhat disappointing in their lack of uniformity, though they do throw some light on the problem.

The failure to obtain more nearly concordant results proved disconcerting at first, but the explanation of the discrepancies is probably to be found in the differences in the behavior of the several digitalis principles, and more especially between that of digitoxin and the rapidly eliminated substance.

When a fatal dose of digitoxin is injected intravenously into the cat, death is delayed during a period varying from a few hours to a day or two, though the heart stops with extraordinary suddenness—often within a few seconds—following the intravenous injection of a massive dose of digitoxin. From this it follows that the more slowly digitoxin is injected intravenously the smaller is the total amount required with continuous injection to cause death, but, as previously indicated, much more of this rapidly eliminated substance is required apparently when the injection is made slowly and continuously, than when the injection is made rapidly, till death occurs, because elimination removes more of a rapidly eliminated substance during the period of slow injection than during the shorter period required with rapid. Obviously, then, the behavior of a mixture of the two types of digitalis bodies with reference to the amounts required will vary somewhat with the relative amounts of the two which are present. There can be little doubt that this factor enters into the explanation of the variable results observed.

It was, therefore, sought to compare the maximum toxicity of digitalis (tincture) with rapid injection, usually within a period of twenty minutes to one hour, with the minimum toxicity with slow injection, usually within a period of three to six hours. It was often impossible to maintain the duration of the experiments and the rate of injection as designed, for reasons which become apparent from a study of the problem.

The estimation of the maximum toxicity with rapid continuous injection involves an unavoidable error, since death does not occur until several minutes have elapsed after the injection of the minimal fatal dose. During this interval before death the superfluous amount

injected will, of course, depend on the rate of injection, and the more rapid the injection the greater will be this excess over the amount required.

It was thought to minimize this error by injecting a supposedly nearly fatal dose at once, and continuing the injection at a rate somewhat slower than would be required to administer the total dose within the period designed for the experiment, with a uniform rate of injection.

The minimum toxicity (within the period allowable for the experiment) was determined by injecting an initial dose designed to be nearly fatal, and after an interval of some hours injecting small amounts at short intervals, or a dilute solution continuously until the heart stopped, or, more accurately, until symptoms indicated that a fatal dose had been administered. In two experiments in which the minimum toxicity of the English leaf was determined, the "combined ouabain" method described by Hatcher and Brody [4] was employed.

In some instances, the initial dose proved fatal, and while such experiments are not without value in determining the maximum toxicity of the specimen, they do not indicate just how far the minimum fatal dose was exceeded. When the animal died within a few minutes following such a single injection, it was considered an indication that the dose was much above the minimum required for that animal, not necessarily much above the average, since the animal may have been unusually susceptible; but when death was delayed for as much as fifteen to twenty minutes, it was accepted as an indication that the dose was not much above the minimum required to cause death.

It will be understood from the foregoing discussion that the failure to cause death during rapid administration with a smaller dose than that required by slower injection does not preclude the presence of at least small amounts of the rapidly eliminated substance, since the unavoidable error inherent in rapid injections, and the increasing action of digitoxin (if, indeed, there is any free digitoxin present in the leaf), during the longer periods of slow administration, tend to counterbalance the lessened effect of the rapidly eliminated fraction. With some of the specimens used, the evidence points unmistakably to the presence of such a rapidly eliminated fraction; in others the results are not conclusive.

The following specimens of digitalis were selected for examination as being fairly representative of the different kinds of leaf commonly

4. The "combined ouabain" method of estimating digitalis bodies, consists in determining the difference between the average fatal dose of ouabain for the cat per kilogram of weight, and that required to kill after the previous injection of a dose of any digitalis body. The difference represents the percentage of the fatal dose that is attributable to the digitalis body under investigation. This is illustrated in one of the protocols given. For details of the method see the paper of Hatcher and Brody: Am. J. of Pharm. **82**:360, 1910.

used in this country: 1. Allen's English digitalis in No. 60 powder. 2. The leaf of the first year's growth, cultivated in Virginia. 3. Leaf of the first year's growth, cultivated in Minnesota. 4. Specimen of fluidextract assayed in 1912 and found to be active. 5. Specimen of fluidextract assayed in 1912 and found to be much less active than the preceding. 6. Specimen of fluidextract recently assayed and found to be active, but which had been submitted for examination, with the complaint that long-continued administration failed to induce the typical therapeutic effects in man.

Tinctures of the leaves were prepared and these were diluted with a small volume of normal salt solution, or weak alcohol, when initial injections of definite amounts were to be made, and with larger amounts of normal salt solution when the injections were to be made slowly and continuously. Fluidextracts were usually diluted with about an equal volume of 20 per cent. alcohol for initial injections, and with about 99 parts of normal salt solution for continuous injections.

A protocol, in brief, will be given of an experiment showing the determination of the maximum activity with a shorter period, and of one with a longer period, and the results of the several series will be tabulated. The second experiment detailed was one of the two in which ouabain was used for estimating the percentage of the first dose that remained active.

Test of concentrated tincture of English digitalis; after dilution with normal saline 1 cc. represents 4.76 mg. of the leaf.

Female cat, gray; weight 2.72 kgm., ether for operation.—10: 55 to 11: 03 a.m. 9.7 cc. per kilogram (representing 46 mg. of digitalis) into the femoral vein; rate of injection slowed.

11: 57 a.m. Convulsion and death; 15.8 cc. per kilogram injected (75 mgm.); death in 62 minutes after starting.

Male cat, gray; weight 3.84 kgm.—10: 20 a.m., 0.12 cc. concentrated tincture (57 mgm. of digitalis) per kilogram into vein; animal released after closing wound, shows great prostration.

10: 27 a.m. Emesis; repeated.

2: 53 p.m. Start injection of ouabain solution 1: 100,000 slowly into femoral vein.

3: 26 p.m. 0.05 mgm. ouabain per kilogram total injected; convulsion and death.

Calculation.—Since 0.1 mg. ouabain is the fatal dose for 1 kg. weight of cat, 0.05 equals 50 per cent. of the fatal dose, indicating that 50 per cent. of the fatal dose is due to the 57 mg. of digitalis previously injected; hence, 114 mg. per kilogram would be required to cause death with this rate of elimination.

It may be asked why the number of experiments designed to determine the amounts required to cause death in the shorter and longer

TABLE 1.—THE TOXICITY OF DIFFERENT SPECIMENS OF DIGITALIS WITH DIFFERENT INTERVALS FOLLOWING THE INITIAL INJECTION AND BEFORE THE DEATH OF THE ANIMAL *

Initial Dose per Kilogram Mg.	Interval Minutes	Total Dose per Kilogram Mg.
	English Leaf	
62	7	65
48	33	56
46	62	75
62	299	127
57	306	114
	Virginia Leaf	
70	20	86
70	20	86
60	23	66
70	23	98
50	27	75
85	41	100
85	56	106
51	58	111
60	70	116
80	306	112
50	384	114
65	397	108
	Minnesota Leaf	
65	10	65
65	23	76
65	40	70
65	99	97
70	231	90
70	253	82
75	256	86
65	310	91
60	327	103
50	360	98
65	370	86
65	418	99
	Fluidextract of 1912	
continuous	15	98
110	20	110
continuous	59	116
100	89	110
70	326	142
120†	335	150
70	368	138
70	371	163
70	372	110
	Fluidextract (Poorer Specimen)	
continous	13	165
160	33	175
160	72	244
160	78	270
160	89	338
160	90	277
100	291	253
130	310	220
130	317	256
130	348	169
100	363	263
	Fluidextract (active, but clinically unsatisfactory	
120‡	4	120
120‡	10	120
100	29	100
95	85	105
100	91	105
120	323	150
100	359	138
110	368	122
100	368	124

* The doses are expressed in milligrams of digitalis represented in the tincture or fluidextract administered; the intervals are given in minutes. Fractions are omitted.

† This animal was evidently tolerant, but the inclusion of the result does not materially affect the average.

‡ These initial doses were too large, as shown by the rapidity of the onset of death; a dose of 100 mg. represents the average minimal fatal probably, though a dose of 120 mg. was survived for some time in another experiment.

TABLE 2.—AVERAGES OF FATAL DOSES OF THE SEVERAL SPECIMENS, WITH THE AVERAGES OF THE SHORTER AND THE LONGER INTERVALS BEFORE DEATH

	Average Time of Interval		Average Dose per Kilogram Mg.
	Interval	Time Minutes	
English leaf	Short	34	64
	Long	302	121
Virginia leaf	Short	26	85
	Long	212	111
Minnesota leaf	Short	24	70
	Long	291	92
Fluidextract 1912	Short	31	108
	Long	310	137
Fluidextract (poor)	Short	23	170
	Long	217	254
Fluidextract	Short	14	113
	Long	266	124

periods respectively varies so widely. It was supposed that the duration of the action of the rapidly eliminated substance varies from three to five hours, and that the elimination would be roughly proportional to the interval elapsing after the initial injection and before death. It now seems probable, in the light of further experience, that the rate of elimination varies rather widely in different individuals.

The periods in which death followed after an interval of an hour or less are chosen, somewhat arbitrarily, as the shorter periods, and the remainder for the longer periods. One experiment of sixty-two minutes is included in the shorter series, but the ratio is not altered materially thereby.

One other specimen of digitalis was included in the study, but the results with it have been discarded because the maximum toxicity for the shorter period was not determined satisfactorily, owing to an oversight. One animal—evidently more susceptible than the average—died within a few minutes following a small initial dose, and it would be misleading to accept this dose as representing the average maximum toxicity of the specimen. There was no important difference between the amounts of this specimen required to cause death with intervals varying from 116 to 489 minutes. Though this series is discarded, as stated, the results, so far as they can be accepted, support the results obtained with the other specimens.

It is possible that the difference shown between the amounts of the English specimen required with rapid and slow injection was greater than would be found with a larger series of experiments, but this specimen was very active, as shown by the biologic test as well as by therapeutic use, and it is possible that it contains a large proportion of the rapidly eliminated substance.

There is no doubt that the averages of the amounts given in the shorter periods are somewhat too high for the reasons already stated, i.e., the difficulty of avoiding the injection of too much when the time allowed for the injection is very short. The first experiment with the poor specimen of fluidextract affords an illustration of this. The injection was continuous and a total of 165 mg. per kilogram of weight were injected within thirteen minutes. It is difficult to avoid the conclusion that all of the drug injected during the last two or three minutes was above the minimal requirement. In the absence of data on which to base a calculation of such errors we are forced to use the figures obtained.

The results with the last fluidextract are somewhat surprising in view of the fact that it was sent to the laboratory for examination because of its failure to induce the characteristic effects in man with long-continued administration. It is more than possible, however, that

the rate of elimination in man is quite different from that in the cat. This specimen was used later by Dr. Cary Eggleston, who observed prompt therapeutic effects following its use by the high dosage method, in which, however, the total amount administered to the patient within a period of about twenty-four hours was only about one-eighth of that administered to one without notable effects over a very much longer period, and which resulted in the complaint just mentioned (oral communication).

These results support the view that the difference between the amounts actually required by rapid and slow injection is greater than that shown in Table 2, and therefore that the initial doses of 120 mg. given in the table were too large.

It is regretted that it was impossible to determine the percentage of the rapidly eliminated substance in the several specimens examined, though there is no reason to doubt that a readily eliminated substance, having a true digitalis action, exists in the leaf, and one must conclude that it is associated with the chloroform-soluble substance with which Hatcher worked. Its action becomes more prominent when separated from the larger proportion of active principles in the leaf, thus explaining why Hatcher has been able to observe evidences of its presence in his preparation so much more constantly than I have been with the tinctures and the fluidextracts of the leaf.

SUMMARY

Seven specimens of digitalis were examined in order to determine the difference between the amounts required to cause death following the intravenous administration in the cat in short periods as contrasted with the amounts required in longer periods of administration.

Evidence is submitted to show that the total amount of digitalis required in this way to cause death is less when this amount is injected within a few minutes than that required when the administration is prolonged over a period of several hours. This is the opposite of the case with digitoxin, of which more is required to cause death promptly than after the lapse of some hours.

The evidence that we are dealing with a body having a true digitalis action is afforded by the fact that it is synergistic with other digitalis bodies, such as ouabain.

The results obtained are interpreted as indicating the presence in the leaf of a digitalis body having a shorter period of cardiac action in the cat than that of any true digitalis body hitherto studied in this way. This brief period of cardiac action is regarded as evidence for its "elimination" in the sense previously defined.

There is some clinical evidence in support of the view here expressed.

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THE CLEAN INUNCTION TREATMENT OF SYPHILIS WITH MERCURY

PRELIMINARY REPORT *

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Inunctions have been used in the treatment of syphilis since the earliest times. In using them, the usual custom has been to leave the inunction on the part of the body to which it has been applied. The recommendation to leave the excess of ointment on the skin does not seem to have been based on actual comparisons, but on the theoretical views as to the mechanism of mercury absorption; i. e., that the mercury is absorbed mainly by inhalation, and that it may be absorbed directly through the stratum corneum of the skin.

The experiments of Wile, Elliott, Schamberg, Kolmer, Raiziss and Gavron speak strongly against inhalation as the essential mechanism. The second explanation was shown to be erroneous by the work of Neumann and Fuerbringer, who many years ago showed that, after rubbing mercury ointment into the skin of men and animals, mercury globules could be found in the hair and sebaceous follicles to about two thirds of their depth. None was found in the sweat glands except at the orifices. They concluded that these globules must be taken up from these parts, as in a few days they were decreased in number and in a few weeks entirely gone. While soluble mercury was to be found chemically in the internal organs, mercury

* From the Department of Dermatology and Syphilology of the Cleveland City Hospital and of the Western Reserve University School of Medicine, and from the Department of Pharmacology and Therapeutics of the Western Reserve University School of Medicine.

globules were nowhere to be found internally. They concluded that the absorption took place from the rubbed places alone, for even by perfectly closing off all the lungs from this territory, mercury was found in the body. They also found that the unperforated epidermis does not absorb mercury, and concluded that the absorption of mercury took place by the influence of sodium chlorid, fatty acids and albuminates, especially through the influence of the gland secretions of the skin. If absorption is limited to the mercury that has been rubbed into the glands, the excess of mercury ointment that remains on the surface of the skin, after the rubbing is completed, can serve no useful purpose.

This gives us a valuable point in therapeutics for several reasons. The inunctions are not used by many: (1) because they are dirty and disagreeable; (2) they are liable to lead to discovery, and (3) when the preparation remains on the skin for such a length of time it is more liable to set up a folliculitis. Now, if the patient, after a thirty minute rubbing, can cleanse off what is left on the skin, none of these points come into consideration and we have an avenue of approach in the treatment of syphilis that can be used by the patient himself without fear of discovery, with no uncleanliness, with no irritation to the stomach, and with none of the attendant pain that comes from the use of mercury injections. We have felt this to be such an important consideration that we have attempted to prove our point from the clinical standpoint. For this purpose we took at random a series of forty-four patients in all stages of syphilis, though most of them were in the secondary stage.

With these patients the following technic was used under the eyes of an orderly: Four grams of the official Unguentum Hydrargyri U. S. P. was rubbed in for thirty minutes. At the end of this time all mercury remaining was thoroughly removed from the skin by the orderly by the free use of benzin and cotton. With these patients a different spot was used each night for at least six nights in order to prevent chances of irritation of the skin, and so that criticism could not be raised that mercury was being absorbed through the irritated skin. The patients were watched primarily with the desire of seeing how soon they would show

symptoms of hydrargyrism, or, rather, if they would show symptoms of hydrargyrism with the same number or rubs as the average individual on whom the inunction was allowed to remain. Secondarily, a certain number with secondary syphilis were observed to note the therapeutic action of the mercury inunction following this technic. Out of forty-four patients we succeeded in getting a marked salivation and edema of the gums thirty-two times. These were the average run of patients that one sees in hospital practice, each patient receiving the routine potassium chlorate mouth wash and tooth brush treatment twice a day, the number of inunctions being 20, 17, 9, 10, 13, 5, 8, 9, 7, 17, 19, 23, 10, 13, 13, 13, 15, 17, 14, 12, 17, 18, 6, 8, 13, 13, 11, 19, 13, 13 and 16. In nine, the salivation and edema of the gums were not so marked, the number of inunctions being 9, 15, 17, 6, 18, 10 and 14, while in five there was extreme salivation and edema, probably in one of the cases being due to a special susceptibility of the drug, as it came on after the sixth inunction, while with the other patients it resulted from 22, 12, 15 and 9, respectively. For the entire series of forty-four patients the number of rubs required to produce stomatitis ranged from five to twenty-three, with a median of fifteen rubs.

By any one doing syphilis work and using mercury inunctions, it must be seen that these figures are practically the same as one usually sees in treating patients with mercury inunctions and leaving the ointment on the body after the rubbing. The average patient is usually able to take from ten to twenty inunctions before symptoms of hydrargyrism—depending partly on the susceptibility of the individual. However, the same results can be achieved, as our figures show, when the drug is cleansed from the skin after the inunction. We believe that these data show conclusively that the full effect of the ointment is secured if it is rubbed thoroughly into the skin for thirty minutes and the excess then cleansed off immediately.

If the mercury is absorbed so as to produce salivation, it must also produce the therapeutic effects of the drug. The occurrence of salivation is, of course, convincing proof of the absorption of therapeutic quantities of mercury. However, we confirmed this directly. In

several of these cases with secondary eruption we did not give any arsphenamin at first, merely using the inunctions. Our first patient, a man with secondary syphilis and mucous patches, showed a positive therapeutic result after twenty rubs, with resultant salivation and slight edema of the gums. The next patient, with a maculopapular eruption, received eight rubs, when he became salivated so badly that we had to begin the use of arsphenamin and stop the mercury, at which time it had as yet no especially positive effect on the disease. One patient, a colored man with a marked lichen syphiliticus, and fairly good teeth, had a positive result after seven rubs, at which time he was salivated so badly that we had to stop the mercury and begin arsphenamin; while another patient, a colored man with a follicular syphilid and good teeth, had a positive therapeutic result after seventeen rubs, when his gums were so badly affected that we had to discontinue the drug and use arsphenamin. Still another colored patient with a follicular syphilid was likewise salivated at the end of seventeen rubs, when we had to stop the mercury. He had had a very good result from the mercurial inunctions. This was also true of a white man with a maculopapular syphilid after eighteen rubs; while another man with a primary lesion and good teeth was salivated after six rubs so that we had to stop the mercury, the therapeutic result, of course, being as yet slight. To be sure, these patients were not cured of their syphilis by these few mercury rubs. Nevertheless, the symptoms of their disease were much benefited—certainly as much as when using inunctions according to the old technic.

CONCLUSIONS

As a result of our preliminary clinical study we believe we are justified in drawing the following conclusions:

1. In treating syphilis by means of mercurial inunctions, probably the only mercury absorbed is that part of the mercurial ointment which is rubbed into the hair follicles, and entrances of the sebaceous and sweat glands. Hence, all superfluous ointment remaining on the skin may be cleansed off immediately after the inunction without lessening the mercurial effect.

2. From forty-four clinical cases of syphilis treated by this technic, we feel that we have been able to prove this clinically.

3. As a result of our findings we feel that, in the future, mercurial inunctions need not be discarded because of the unpleasant considerations in regard to their use, namely, uncleanliness, liability of discovery, and causing of a folliculitis.

4. Mercurial inunctions following the technic that we advise are to be recommended in the treatment of syphilis as a distinct advance in the therapy of this disease.

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THE PREVENTION OF SIMPLE GOITER IN MAN *

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Simple or endemic goiter is one of the most benign and insidious diseases of man and animals. The sum total of its ravages throughout all ages and in all lands is still unrealized by the public generally, notwithstanding the numerous reports of commissions appointed for its study. Those who live on the sea coasts fortunately have had no need to be concerned; and those who lived in goiter districts—before the days of extensive travel—grew accustomed to look on goiter as natural and normal. Indeed, in many districts of the world, it is still looked on as a mark of beauty.

Simple goiter includes all those thyroid enlargements in man and animals formerly grouped as endemic, epidemic, sporadic and physiologic. It must be sharply distinguished from exophthalmic goiter, with which it has no necessary association or etiologic relationship. Exophthalmic goiter, so far as is yet definitely known, occurs spontaneously only in man, while simple goiter occurs in all animals having the ductless thyroid. Exophthalmic goiter is not notably associated with districts, while with simple goiter this is most characteristic. Exophthalmic goiter occurs more frequently in the highly developed and civilized races, while in simple goiter race is not a factor. Simple goiter may develop

* From the Laboratories of Western Reserve University, Cleveland, and Montefiore Hospital, New York.
* Read before the Section on Pharmacology and Therapeutics at the Seventy-Second Annual Session of the American Medical Association, Boston, June, 1921.
* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

sporadically in any place (even at sea, as reported on one of Captain Cook's voyages), but it is preeminently associated with certain regions or districts. The distribution of these districts of endemic goiter throughout the world was fully described by Hirsch, in 1860. The actual incidence of goiter within a given district is still quite unknown. With the information at present available, however, one can distinguish between mildly and severely goitrous districts. As compared with certain other districts, for example, the Alps and the Himalaya regions, our most important districts, namely, the Great Lakes Basin and the Cascade Mountain regions of Oregon, Washington and British Columbia, would be classified as mildly goitrous. The mildness or severity of a district may be determined by the incidence of myxedema or cretinism—a fact known to Morel and expressed in his famous dictum, "Goiter is the first halting place on the road to cretinism" (Le goître est la première étape sur le chemin qui conduit au cretinisme).

ETIOLOGY

The ultimate cause of simple goiter is totally unknown, notwithstanding a relatively large amount of study. The immediate cause is a lack of iodin. The enlargement, therefore, is a symptom and may result from any factor which increases the iodin needs of the organism, as in certain types of infection, or which interferes wtih the normal utilization of iodin; or it may result from actual experimental deprivation of iodin. The conception that it is due to a contagium vivum in the sense that this term is ordinarily used may be abandoned. Water has been associated as an etiologic factor by all peoples as far back as history goes. The American Indians (Barton) and the natives of Central Africa (Livingston) seem to have been as strongly convinced of the relation of water to the disease as was Hippocrates. If water is a factor, it would seem that it is the absence rather than the presence of some substance, which is to be considered, since goiter is associated with the purest of waters, chemically and bacteriologically as, for example, in Portland, Ore., and in Seattle and Tacoma, Wash., where there has been a rapid increase in goiter since these cities began to take their water supplies from the Cascade Mountains.

After consideration of all the various substances, agents and theories that have been put forward as having a rôle in the etiology of goiter, we at present must fall back on the view that thyroid hyperplasia (goiter) is a compensatory reaction arising in the course of a metabolic disturbance and immediately depending on a relative or an absolute deficiency of iodin.

PATHOLOGIC ANATOMY

Anatomically, a wide range of changes may be present, depending on the species of animal and on the stage (duration) of the disease. It always begins with a decrease in the colloid material and a hypertrophy of the epithelial cells, at first cuboidal, later columnar, with infoldings and plications. In man and fowls, the stage most commonly observed is characterized by an abundance of colloid material—the so-called cystic or colloid goiter of the older writers—while in dogs, sheep, cattle, pigs, fish, etc., the accumulation of colloid is seen only in the late regressive or quiescent stages. In man, the adenomatous form (struma nodosa) is very common, but it is exceedingly rare in all the lower animals. These adenomas, in all probability, arise from fetal cell rests. The stimulus which initiates the growth of the cell rests (adenomas) and that which initiates the growth of the more differentiated thyroid tissue are probably identical. These growths have many of the attributes of tumor, in that their growth may not be arrested by iodin administration or by the natural physiologic compensation.

EXPERIMENTAL PHYSIOLOGY

No accomplishment in preventive medicine has a firmer physiologic and chemical foundation than that underlying goiter prevention, and, as the work of prevention is based on certain of these facts, the more important may be reviewed:

1. The active principle of the thyroid is a very stable organic compound of iodin, first recognized by Baumann, in 1895, and recently (1916) isolated in crystalline form, by Kendall.

2. The developmental stage of all goiters is characterized by an increased blood flow, an increase in the size and number of epithelial cells, a decrease in the stainable colloid, and a marked absolute decrease in the

iodin store. The decrease in the iodin store precedes the cellular hypertrophy and hyperplasia.

3. Similar changes (compensatory hyperplasia) invariably occur in the remaining portion of the gland, when a sufficient portion of the entire gland is removed. The amount of gland it is necessary to remove in order to cause compensatory hyperplasia varies somewhat with the species of animal, definitely, with the age, diet, and the presence or absence of iodin.

4. The administration of exceedingly small amounts of any salt of iodin in any manner completely protects the remaining thyroid against compensatory hyperplasia, even after the removal of three fourths of the normal gland in cats, dogs, rabbits, rats and fowls. Halsted and Hunnicutt reported a series of partial removals in dogs in which they failed to obtain compensatory hyperplasia, while Loeb has recently reported a series of partial removals in guinea-pigs in which iodin failed to prevent the compensatory hyperplasia, although desiccated thyroid still protected. He concluded that regeneration was physiologically different from spontaneous hyperplasia or simple goiter. The explanation for Halsted's results was probably that the animals were in contact with a source of iodin, while the most probable explanation for Loeb's results is that he removed too much thyroid, since, as shown by Marine and Lenhart, in 1909, iodin will not protect even in dogs if more than three fourths of the gland is removed, while desiccated thyroid will protect the animal against thyroid regeneration even after the removal of as much as nine tenths.

5. If most of the thyroid gland is removed before or in the early stages of pregnancy, and rigid precautions are taken to exclude iodin, the young at birth will have enlarged thyroids, as first shown by Halsted in dogs; while, if iodin is available, the young at birth will have normal thyroids.

6. A milligram of iodin, given at weekly intervals, has been found sufficient to prevent thyroid hyperplasia in pups.

7. Thyroid tissue has an extraordinary affinity for iodin, as has been demonstrated in in vitro perfusions of surviving thyroids, and also by injecting intra-

venously small amounts of some soluble salt of iodin into the intact animal.

8. If the iodin store in the thyroid is maintained above 0.1 per cent., no hyperplastic changes, and therefore no goiter, can develop.

The foregoing experimental data seem to us sufficiently complete to demonstrate the underlying principles of goiter prevention, and the ease with which they may be applied. The first instance in which these facts were utilized in the prevention of goiter on a large scale occurred in 1909 and 1910. Working with endemic goiter in brook trout, Marine and Lenhart were able to demonstrate that iodin added to the water in a concentration not exceeding 1 : 1,000,000 arrested or prevented the development of thyroid hyperplasia (goiter). Since then, the method has been successfully applied on a large scale by several observers in the prevention of goiter in cattle, sheep, pigs and poultry.

To our knowledge, the prevention of human goiter was not attempted on any large or practical scale until 1917, when we began work with the school population of the city of Akron, although in Cleveland it had been strongly urged and had been used by some physicians for several years. Briefly, the method as applied to man consisted in the administration of 2 gm. of sodium iodid in 0.2 gm. doses, distributed over a period of two weeks, and repeated each autumn and spring. This amount of iodin is excessive, and far beyond the needs of the individual or of the ability of the thyroid to utilize and store it. One gram distributed over a longer period would be better. The form or mode of administration of iodin is of little consequence. The important thing is that iodin for thyroid effects should be given in exceedingly small amounts, and it is believed that most of the untoward effects recorded are due to the excessive doses employed, or, more concretely, to the abuse of iodin.

The results of our two and one-half years' observations on schoolgirls in Akron are as follows: Of 2,190 pupils taking 2 gm. of sodium iodid twice yearly, only five have developed enlargement of the thyroid; while of 2,305 pupils not taking the prophylactic, 495 have developed thyroid enlargement. Of 1,182 pupils with thyroid enlargement at the first exam-

ination who took the prophylactic, 773 thyroids have decreased in size; while of 1,048 pupils with thyroid enlargement at the first examination who did not take the prophylactic, 145 thyroids have decreased in size. These figures demonstrate in a striking manner both the preventive and the curative effects. Klinger has recently (1921) reported even more striking curative results in the schoolchildren of the Zürich district. He worked with school populations in which the incidence of goiter varied from 82 to 95 per cent., while our maximum incidence in Akron was 56 per cent. With such a high natural incidence of goiter, his observations necessarily deal more with the curative effects. Thus of 760 children, 90 per cent. were goitrous at the first examination. After fifteen months' treatment with from 10 to 15 mg. of iodin weekly, only 28.3 per cent. were goitrous, of a total of 643 children reexamined.

The foregoing results were obtained in adolescents. There are two other periods in life when simple goiter frequently develops, namely, (1)) in fetal life and (2) during pregnancy. While the thyroid enlargements developing around the age of puberty are more common, they are not more important than those developing during pregnancy and fetal life. The higher birth mortality of infants with congenital goiter is well known. The thyroid enlargement of both mother and fetus may be prevented by giving 2 gm. of sodium iodid, or its equivalent in iodin in any other form, during the first half of pregnancy.

UNTOWARD EFFECTS

The dangers of giving iodin, in the amounts indicated, to children and adolescents are negligible. Exophthalmic goiter and iodism are the two important conditions to be looked for. No case of exophthalmic goiter developed in the series reported by Klinger or by us, although in both instances such cases were carefully looked for. Much has been written of iodin-exophthalmic goiters, but a study of the case reports reveals the fact that they resulted from the use of excessive (according to physiologic standards) amounts of iodin, or of desiccated thyroid. In adults, the possibility of aggravating a mild exophthalmic goiter or even the production of the syndrome in susceptible individuals must

be considered. Again, the risk is slight. Iodin should not be given in any frank case of exophthalmic goiter unless the patient can be daily observed, and then it should be administered only in milligram doses. Iodism was observed in eleven cases among the schoolchildren of Akron during the two and one-half years of observation. Most of these cases were very mild, and the girls did not stop the treatment. Klinger did not observe a single instance in sixteen months' observation on more than 1,000 children, although iodism was carefully looked for.

SUMMARY

Simple or endemic goiter in man may be prevented as cheaply and as simply as in the lower animals, by the administration of 3 to 5 mg. of iodin twice weekly, over a period of a month, and repeated twice yearly. Klinger in Switzerland has reported as striking, and nearly as extensive, results as those obtained by us in Akron. In young individuals, with goiter of recent development, the curative effects of exceedingly small amounts of iodin are as marked as one sees in the goiter of animals.

There are no dangers worthy of consideration associated with the administration of the quantities of iodin used by Klinger or by us. Simple or endemic goiter most commonly develops during (1) fetal life, (2) around the age of puberty, and (3) during pregnancy, and we believe that any plan which provides for its control during these three periods of life will practically eliminate endemic goiter. Goiter in the mother and fetus can be prevented as simply as that of adolescence, but, practically, it would seem that it is a responsibility of individual physicians, supplemented by public education. The prevention of goiter of childhood and adolescence should be a public health measure, best administered through the schools in order to combine the important additional factor of education. Beginning with the period of puberty, goiter occurs approximately six times as frequently in females as in males. The question, therefore, whether general prophylaxis should include both males and females would depend to some extent on whether the particular district was mildly or severely goitrous; hence the need for accurate surveys. The age of beginning and stopping the

use of iodin would depend to some extent on race and climate. In the United States, probably the maximum of prevention coupled with the minimum of effort would be obtained by giving iodin between the ages of 11 and 17 years.

The prevention of goiter means vastly more than eliminating cervical deformities. It means, in addition, the prevention of those forms of physical and mental degeneration, such as cretinism, mutism and idiocy, which are dependent on thyroid insufficiency. Further, it would prevent the development of thyroid adenomas, which are an integral and essential part of endemic goiter in man, and due to the same stimulus. These multiple, circumscribed benign growths have many of the attributes of tumor, one of which is that their growth once initiated is frequently not controlled by iodin, as are all simple hyperplasias. The terminal metamorphoses are far more serious than those of simple hyperplasia, since, in addition to hemorrhage, necroses, cyst formation, etc., probably 90 per cent. of the malignant tumors of the thyroid arise from these adenomas.

If the prevention of goiter is good preventive medicine, it is better preventive surgery. With so simple, so rational and so cheap a means of prevention at our command, this human scourge, which has taken its toll in misery, suffering and death throughout all ages, can and should be controlled, if not eliminated.

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ANESTHESIA IN NOSE AND THROAT WORK

FURTHER REPORT OF THE COMMITTEE ON THE ADVANTAGES AND DISADVANTAGES OF THE VARIOUS LOCAL ANESTHETICS IN NOSE AND THROAT WORK *

EMIL MAYER, M.D., NEW YORK, CHAIRMAN; ROSS HALL SKILLERN, M.D., PHILADELPHIA; ROBERT SONNENSCHEIN, M.D., CHICAGO, AND WILLIAM B. CHAMBERLIN, M.D., CLEVELAND, COMMITTEE

Your committee, which was continued from last year, has the honor to report that on Jan. 15, 1921, the following letter was sent to each member of the section who had been registered within the last five years:

Dear Doctor:—At the 1920 Annual Session of the American Medical Association, the section's Committee on the Advantages and Disadvantages of Local Anesthesia presented a report.

The committee was continued and, by a unanimous vote of the section, requested to communicate with and enlist the cooperation of each Fellow who, during the last five annual sessions, has registered in the section, in order that there may be secured further individual experiences regarding toxic effects following the use of local anesthetics.

In accordince with the above you are asked to supply the committee with the following information:

1. Have you during 1919 and 1920 noted any toxic effects, fatal or not, following the use of a local anesthetic? Yes, No.

2. If so, please submit a case report of each instance; record among other facts data regarding the patient's general condition, the occasion for using the local anesthetic, the drug employed and the dose administered.

3. Kindly inform the committee of the name and address of any physician known by you to have had such experience.

* Read before the Section on Laryngology, Otology and Rhinology at the Seventy-Second Annual Session of the American Medical Association, Boston, June, 1921.

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

Appreciating the cooperation you will give the committee by making an early reply, which may be made on the reverse of this sheet of paper, and should be mailed to,

Yours very truly,

WILLIAM B. CHAMBERLIN,
Secretary of the Section.

Fourteen hundred letters were thus sent, with 315 replies.

Question 3, asking for the name and address of any physician known to have had any toxic effects, was the most valuable in the matter of replies.

The alleged deaths thus reported were thirty-two, and in each instance attempt was made toward verification. Five of these were reported as errors, but twenty-seven of them have been substantiated. From twenty-two of these, details were sent by the physicians written to, while five of them have failed to respond in any way, although repeatedly written to in the name of the section and Association, as also in the name of humanity.

Of the twenty-two deaths, eleven were from cocain (three mistakes of nurses), five from procain and cocain, three from procain only, one from apothesin and cocain, one from apothesin only, and one from alypin and cocain. All of these fatalities have occurred within the last two or three years, and with the exception of three, none have been reported in the medical journals.

The amount of epinephrin used was carefully gone into, and in no instance could this preparation be considered as an agent in causing death.

SUMMARY OF DEATHS

A brief summary of these deaths is herewith presented:

COCAIN DEATHS

CASE 1 (mistake).—*Tonsil case.*—Spring of 1919, woman, aged 18. Injection of 4 per cent. solution of cocain substituted by nurse for 1 per cent. procain; one tonsil was removed, mouth could not be opened after that; patient sitting up, convulsions within five minutes, death in twenty minutes.

CASE 2.—*Tonsil operation.*—June, 1920, girl, aged 16. Probable goiter, morphin with atropin with $\frac{1}{60}$ strychnin by hypodermic before operation. Tonsil injected with 0.2 per cent. cocain solution to which 20 minims of epinephrin, 1 : 1,000, was added to each ounce of the cocain solution. Tonsil easily

removed. Second tonsil injected and removed without pain. Patient was told she could walk to her room, but just as she started she turned pale and immediately went into a rigid spasm, then relaxed and was dead. Sitting in chair during operation and injection.

Case 3.—*Nose case.*—July, 1919, woman, aged 23. Cotton soaked in 10 per cent cocain solution pressed fairly dry, and packed in the nose. Packing left for thirty minutes, when patient was brought to the chair for operation. She said she felt sick, fell forward, had a convulsion and in four minutes was dead. Sitting.

Case 4.—*Tonsil case.*—Spring, 1919, woman, aged 50. One-third per cent. of cocain solution with a few drops of epinephrin chlorid used hypodermically in tonsils. Operation consumed fifteen minutes; patient became nervous and irritable before operation was completed. Five minutes later was returned to her room; died on the cart before being lifted to bed. Upright.

Case 5 (mistake).—*Tonsil case.*—Spring, 1920, woman, aged 26. Throat swabbed with 10 per cent. cocain-epinephrin. One per cent. solution of procain was poured into glass with 8 drops of epinephrin solution. Within two minutes patient went into one convulsion after another and died. It was ascertained that a nurse had poured 20 per cent. solution of cocain into the procain solution without the knowledge of any one. Upright.

Case 6.—*Tonsil case.*—November, 1920, man, aged 35. Pharynx painted three times with 10 per cent. cocain solution. A small amount of 0.2 per cent. of cocain with 5 minims of epinephrin solution, 1:1,000, injected. After the right tonsil had been seized and a small cut made with scissors, patient became pale, seemed fainting, became unconscious, had convulsive spasms, and although every attempt at resuscitation was made, patient was pronounced dead at the end of three hours. Symptoms of distress within two minutes of injection; patient was sitting up during injection. Postmortem: arteriosclerosis of coronary arteries and obstruction as contributary cause, and acute dilatation of the heart.

Case 5 (mistake).—*Tonsil case.*—October, 1919, woman, aged 27. Ten per cent. solution of cocain injected by mistake of nurse for 1 per cent. procain. No operation performed. Toxic symptoms immediate; death did not ensue until eight hours later, during which time there was a period of rallying. Patient sitting up.

Case 8.—*Larynx case.*—August, 1920, man, aged 28.—Papilloma vocal cord. Throat sprayed with a 10 per cent. solution of cocain. Papilloma removed. No toxic symptoms during and immediately after the operation. Patient went home. One hour later returned complaining of feeling faint; convulsions fifteen minutes later and death. Postmortem showed all organs normal.

CASE 9.—*Cystoscopy.*—August, 1920, man, aged 36. Eight cubic centimeters of 4 per cent. solution of cocain injected into the bladder. While cystoscopy was being performed he complained severely of pain, the instrument was removed, convulsions followed, simulating epileptic convulsions; cyanosis and death.

CASE 10.—*Tonsil case.*—September, 1919, woman, aged 18. One-sixth grain of morphin injected one-half hour before operation. Brought to operating room in wheel chair. Injection of about 30 drops of a 2 per cent. solution of cocain-epinephrin, a smaller amount being used on the left than on the right side. Right tonsil removed; patient said she felt queer; was placed on the table and allowed to inhale aromatic spirit of ammonia. She said she felt better. Without further infiltration of the left tonsil this was removed, and this was not so painless. At the end of the operation of the left tonsil, her eyes began to turn toward the right and upward, with a jerking, twitching of the face, then involving extremities, of a severe type. Patient became cyanotic. This lasted about one minute, when she went into a deep coma; from this she rallied, when there was a recurrence of spasm, followed by coma. These repeated themselves about every three or four minutes, and at no time was she clear in her mind between the attacks. Death ensued. There was a suspicion of epilepsy in this patient.

CASE 11.—*Nose operation intended.*—Man, aged 25. Deviated septum. In the office, septum brushed with 1:1,000 solution of epinephrin; five minutes later cocain powder made into mud with epinephrin, rubbed over septum. Five minutes later remaining tender spots on septum touched with same cocain preparation, and patient placed on couch for ten minutes. He then walked to operating chair not more than ten steps when he said he felt faint, was assisted to the couch, had a slight convulsion, and death ensued.

MIXED DEATHS—COCAIN AND PROCAIN

CASE 1.—*Tonsil operation.*—May, 1920, girl, aged 16. "Cocain mud" was applied to pharynx with patient sitting upright. This was followed by injection of 0.5 per cent. solution of procain, 10 c.c. in all used. The procain solution had been prepared four days previously. Two or three minutes after injection, patient had a convulsion, following which all the muscles of the body were taut, eyes rolled upward, head jerked back, and she became markedly cyanotic; patient had marked air hunger, and died one hour and ten minutes after administration of anesthetic. Patient upright.

CASE 2.—*Tonsil case.*—September, 1920, woman, aged 26. Throat swabbed with 20 per cent. solution of cocain, to which 10 to 15 drops of epinephrin to the ounce were added. Ten minutes later injection of 1 per cent. solution of procain, using about 3 drams on each side. Fifteen seconds after last

injection, patient had convulsions with marked cyanosis. In one hour patient was pronounced dead. Postmortem showed no abnormality, except the thymus, which weighed 50 gm. Status lymphaticus; patient sitting up during injection. No operation performed.

Case 3.—*Tonsil operation.*—September, 1920, woman, aged 25. Throat swabbed with a 10 per cent. solution of cocain. Left tonsil injected with 1 per cent. solution of procain. Following this, right tonsil injected, when patient went into convulsions and died in about ten minutes. Patient upright.

Case 4.—*Tonsil case.*—July, 1918, girl, aged 15. Two applications to pharynx of a 10 per cent. solution of cocain swabbed. Injection of 2 drams of 0.5 per cent. solution of procain. After three minutes, left tonsil operated on and it was nearly freed; and as it was finally removed, patient jerked her head back as if she was about to have some sort of seizure. Convulsions followed. Death ensued within a very few minutes, by the time she was placed on the operating table. Patient sitting up during injection and operation.

Case 5.—*Tonsil case.*—March, 1921, man, aged 22. Operated on one year previously for appendicitis under ether. Morphin, ¼ grain, with atropin, 1/50 grain, injected half hour before. Throat swabbed with 10 per cent. solution of cocain twice at five minute intervals. Tonsillar infiltration with 0.5 per cent. solution of procain with 2 drams to each tonsil, one drop of a 1 : 1,000 solution of epinephrin in each dram. As soon as the second tonsil was injected, patient was nauseated and vomited; two minutes later had a convulsion; never regained consciousness. Death in twenty minutes. This was the second operation using the same solution. In the first case immediately preceding there was no toxic symptoms whatever. Patient upright.

PROCAIN ONLY

Case 1.—*Tonsil operation.*—September, 1920, woman, aged 29. Injection of a 0.75 per cent. solution of procain with 1 drop of epinephrin solution to each cubic centimeter. Slowly 5 c.c. was injected on the right side. She complained of vertigo with some precordial distress, which soon passed away. Left tonsil was then injected slowly; when possibly 3 or 4 c.c. had been injected, she complained of vertigo with violent retching and inability to swallow, immediate cyanosis, collapse and unconsciousness. From fifteen to twenty minutes from the time of collapse, no heart movements could be detected. Respiratory paralysis was the first significant symptom. Total amount of procain used less than 1 grain. Patient sitting up.

Case 2.—*Tonsil operation.*—December, 1920, man, aged 24. Five-tenths per cent. solution of procain, 12 c.c. in all, with 9 drops of epinephrin solution were injected. Three minutes thereafter convulsions followed and death ensued. Patient sitting up during entire time.

CASE 3.—*Tonsil case.*—June, 1920, man, aged 36. Four drams of a 2 per cent. saline solution of procain used. Toxic symptoms within four minutes of injection, convulsions and cessation of respiration. Patient reclining in a dental chair. One-sixth grain morphin injected half hour before injection of procain. No cocain whatever was used.

APOTHESIN AND COCAIN DEATH

CASE 1.—*Tonsil case.*—May, 1920, woman. One-eighth grain of morphin given in doctor's office; tonsils swabbed with a 4 per cent. solution of cocain, using two applications. Five minutes later injection of 2 drams of a 0.25 per cent. solution of apothesin. This remained for ten minutes. Just as the first incision was about to be made she fainted and was carried to the recovery room. Heart never responded. Patient had taken enormous amounts of coal tar preparations within the last five years. Was sitting up during injections and operation.

APOTHESIN DEATH [1]

CASE 1.—*For Gastrostomy.*—August, 1920, man, aged 43. Because of dysphagia, due to tuberculosis, gastrostomy was advised. On account of profound cachexia and unfavorable pulmonary condition, spinal anesthesia was advised. Puncture in second lumbar space. Seven c.c. spinal fluid withdrawn, and added to a solution of apothesin in which 1¼ grains of apothesin was dissolved; at the end of three minutes patient became ashy, complained of tightness in the chest, announced that he was dying, respirations became labored and ceased. The author has little doubt that apothesin was indirectly responsible, because of the very definite and rapidly ascending paralysis, culminating in cardiac and respiratory failure, which could be due only to diffusion into medullary centers. It seems also to be clear that cardiac failure preceded a respiratory failure. Patient sitting while being injected.

ALYPIN AND COCAIN DEATH

CASE 1.—*Tonsil case.*—March, 1920, woman, aged 19. Throat swabbed with 10 per cent. cocain solution. About 2 drams of alypin solution, strength not stated, injected. Epileptiform convulsions immediately ensued; all attempts to resuscitate failed, and in half an hour she was pronounced dead. Patient sitting up during injection.

Of these twenty-two deaths, 14 were in females, eight in males. One occurred in 1918, five in 1919, fourteen in 1920, one in 1921, and one date was not given.

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1. Duboff, W. S.: Death from Apothesin Spinal Anesthesia, J. A. M. A. **75**: 605 (Aug. 28) 1920.

It will be noted that we have made a new classification of deaths occurring when both cocain and the synthetic drugs were used in the same patient.

We do not feel that these should be placed solely as occurring after the use of the synthetic drug, especially when the latter was used in very low percentages.

Experiments have shown (Hatcher and Eggleston) that all of the local anesthetics are quantitatively synergistic, so that if one injects 50 per cent. of the fatal dose of each of any two of these at once, the effect is the same as that of injecting 100 per cent. of the fatal dose of one of them.

There is one remarkable case, Case 8 of the cocain series, in which the throat was sprayed with a 10 per cent. solution of cocain, a papilloma removed, the patient went home, returning one hour later feeling faint, when convulsions and death supervened.

Of the cocain deaths, seven patients were injected hypodermically, one had an injection in the bladder, one spray in the larynx and two had packing in the nose. Three of these were due to avoidable mistakes.

The cocain-procain deaths had the former drug applied in strengths varying from 100 to 20 and 10 per cent., respectively, just prior to the injection of the procain.

The procain deaths, three in number, had the solutions injected in 0.75, 0.5 and 2 per cent., respectively.

In the cocain-apothesin death there was a preliminary swabbing of a 4 per cent. solution of cocain, followed by the injection of the apothesin solution.

In one apothesin death the patient was so far advanced in tuberculosis that any slight shock would have killed him, and we are not inclined to accept the death as being solely due to apothesin.

In the cocain-alypin death, a 10 per cent. cocain solution was first applied.

Deaths from this drug are not more frequently recorded because alypin has been so rarely used in this country. In our previous report we called attention to this drug as being very highly toxic, more than twice as toxic as cocain, and it is moreover the least efficacious of all synthetic preparations.

In practically all of the cases convulsions occurred within three minutes of the administration of the local

anesthetic, and death ensued anywhere from twenty minutes to several hours afterward. In none was there any return to consciousness from the first symptom, although every known method of resuscitation was used in each instance.

In our previous report of twenty-one deaths, fifteen followed the administration of cocain and six of procain. In our present series eleven were from cocain, eight from procain, with and without cocain, two from apothesin and one from alypin, showing an apparent increase in the number of deaths from local anesthetics, and it is interesting to note that deaths from these drugs are here reported as having occurred more frequently in 1920 than in any previous year. This observation may be due to our more rigid investigation.

POSITION OF PATIENTS

Nineteen of these were in the upright position, three reclining or semi-reclining.

In our former report we recommended the reclining posture throughout, from the beginning of the use of the anesthetic, and in this connection call attention to a report of toxicity received from one of our members and a careful observer.

> Man, aged 36. Tonsillectomy. One per cent. procain solution injected, to each ounce of which was added 10 drops of the epinephrin solution. Right tonsil anesthetized and removed before anesthetizing left. I then started to inject the left tonsil, made one injection, and the patient suddenly became unconscious, developing right-sided hemiplegia, and remained this way for twelve hours. Respiration embarrassed, resembled Cheyne-Stokes slightly. Patient recovered fully. Position of patient during operation on a table, semi-reclining; previous administration of morphin, ⅙ grain, with atropin 1/150 grain; amount of anesthetic used, 4 drams and approximately 5 drops of epinephrin solution. No cocain used.

May we not venture the opinion that the position of the patient, at the time when the toxic effect was the greatest, relieved all strain of the heart and enabled it to carry on, in spite of the effect of the poison on his system?

This question of position of the patient is very firmly held by those who have followed the reclining method for years, and there seems to be a reasonable amount of logic in its favor.

We have no real conception of time when it comes the seconds, and can value it only as we do in a slow motion picture of a running horse. In this the action, which ordinarily flashes by so rapidly that we cannot express it in terms of time, now shows every detail. May we not by analogy feel that the heart's action in these toxic cases is tremendously affected in that flash of a second, and contend that the relief from all strain of that organ may help it tide the patient over when overwhelmed by the poison?

ALKALINE SOLUTIONS

In our previous report we called attention to the alleged advantages of adding 0.5 per cent. solution of sodium bicarbonate to these local anesthetics, but we have no evidence that this method has been tried.

SUMMARY

We have now given this most important subject closest study during the last two years, and the forty-seven cases that we have been able to compile is the best answer to the query of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

Our own investigation has been limited to members of the section, and the majority of cases coming to our knowledge have been in throat and nose cases.

We can deduce, therefore, that there are many more fatalities occurring among surgeons generally, among other specialists, as also among dentists, and we believe it necessary that there should be further investigation of these occurrences and that some definite action should be taken toward the prevention of fatalities.

We report again one fourth of the cocain deaths due to avoidable mistakes. We also note the varying strength used of this powerful drug, but we do not feel that recommendations as to doses, stringent rules governing the handling of these powerful drugs by totally irresponsible persons, as in the cases marked "mistakes of nurse," or regulations for testing these drugs before being placed on the market, should be made by any committee of a section of the American Medical Association.

The question of having ampules made by the manufacturer containing solutions of synthetic drugs used has also been favorably considered by this committee.

SUMMARY OF RESULTS OF INVESTIGATION OF FATAL CASES FOLLOWING THE USE OF LOCAL ANESTHETICS

Deaths from local anesthetics for last two years*	27
April 1, the committee has received answers to letters inquiring as to foregoing deaths from 22. These are:	
Cocain (3 mistakes of nurse)	11
Cocain and procain	5
Procain only	3
Apothesin and cocain	1
Apothesin only	1
Alypin and cocain	1
	22
No response from members repeatedly written to	5
Total deaths, 1919-1921	27

* These are reported to the committee as a result of 1,400 personal letters from 315 members; only three of those were recorded in medical journals.

CONCLUSIONS

A study of these untoward happenings enables us to reach the following conclusions:

1. Deaths from the administration of local anesthetics are vastly in excess of the number reported in the medical journals.

2. In most instances convulsions are the first indication of toxic effects; consciousness is never regained and death ensues within a comparatively short time.

3. The customary dosage of local anesthetics varies from small amounts to very large ones.

4. There is no check on the manufacturer as to the comparative toxicity of the various batches of drugs that are placed on the market.

5. The freedom from ill effects noticed by so many who have used these drugs has made them oblivious to the likelihood of danger.

6. The presumption of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association, that there are many unrecorded deaths, is thoroughly substantiated.

7. The appointment of a commission to investigate further these deaths and take action thereon is vitally necessary.

RECOMMENDATIONS

We unanimously recommend that this report be sent at once to the chairman of the Council on Pharmacy and Chemistry of the American Medical Association, with the request that he secure the consent of the Board of Trustees to the appointment of a commission, consisting of men active in various fields of medicine, who shall investigate the question of toxicity of local anesthetics; who shall also determine the causal factors of deaths as far as is possible, and report what measures shall be adopted by the medical profession to prevent occurrence of these fatalities, the report of this commission to be made to the Therapeutic Research Committee of the American Medical Association, and when endorsed by the latter referred to the Board of Trustees and the House of Delegates for official action. Finally that this committee be discharged.

Your committee feels that this subject is a vastly important one and although no member thereof has been unfortunate enough to have witnessed a fatality, yet it is very much impressed by the reports of these dreadful occurrences, and deeply sympathize with its colleagues who have had such misfortunes. We take this occasion to express our sincere thanks to those twenty-two members of the profession who have answered our queries and given detailed accounts of fatalities, and regret that the length of this report does not permit a detailed account in each instance. Many of their reports contain data as to the full examinations that have been made of their patients before anything had been attempted, as also detailed accounts of measures used to resuscitate, which for lack of space we feel compelled to omit.

As in our previous report, we express our appreciation of the encouragement and assistance of Prof. Torald Sollmann, chairman, and Prof. Robert A. Hatcher, and the Therapeutic Research Committee, the latter voting the payment of the actual expenses of this committee in assembling and preparing this report.

Respectfully submitted,

EMIL MAYER, *Chairman*,
ROSS HALL SKILLERN,
WILLIAM B. CHAMBERLIN,
ROBERT SONNENSCHEIN.

Reprinted from Transactions of Section on Ophthalmology of the American Medical Association, 1921

REPORT OF COMMITTEE ON LOCAL ANESTHETICS IN OPHTHALMIC WORK*

To the Members of the Section on Ophthalmology, American Medical Association:

About three years ago, the Council on Pharmacy and Chemistry of the American Medical Association decided to ascertain the advantages and disadvantages of the various agents used for producing local anesthesia for general as well as specific purposes, and to decide which local anesthetics have distinct usefulness, which are objectionable, and which are superfluous duplications. In carrying out this purpose, a series of investigations was conducted by the Research Committee of the Council, which embraced a study of the toxicity, activity and efficiency of the local anesthetics as determined by experimentation on animals. These studies are contained in a number of papers published in the Therapeutic Research Reports of the American Medical Association and form a trustworthy and valuable addition to our knowledge of the comparative value of the local anesthetics.

A little more than a year ago, the Council requested that this Section aid in the clinical investigation of the subject, and a committee was appointed to study the subject of local anesthetics in ophthalmic practice.

The committee decided to draw deductions from the following:

1. A survey of the acquired experience of representative ophthalmologists.
2. Clinical investigation.
3. Experimental investigation on animals.

* Read before the Section on Ophthalmology at the Seventy-Second Annual Session of the American Medical Association, Boston, June, 1921.

4. An analysis of the literature.

In carrying out the first purpose, your committee sent a questionnaire to all of the prominent members of this Section.

The questions asked were:

1. Which local anesthetics do you use at present, and in what strengths?

2. Do you use one local anesthetic for all classes of cases? If not, give particulars.

3. What special reasons have you for your preference as to the local anesthetic, strength and cases?

4. Have you abandoned or decreased the use of any anesthetics that you have tried? Give reasons.

5. Name, and state briefly, your experience as to the following points for the local anesthetics which you have tried. (Where more than one anesthetic has been used extensively, explain each in detail.)

(*a*) Agents and strength or concentration for superficial anesthesia.

(*b*) Agents and strength of concentration for deep anesthesia.

(*c*) Time of onset and duration in both cases.

(*d*) Degree of irritation, both cases.

(*e*) Effect on vascularity.

(*f*) Effect on pupils.

(*g*) Effect on corneal epithelium.

(*h*) Effect on accommodation.

(*i*) Effect on intra-ocular tension.

(1) Normal.

(2) Glaucoma.

(*j*) Occurrence of systemic intoxications.

(*k*) Stability of solution, including effect of sterilization by boiling.

(*l*) Effect of addition of epinephrin on intensity of anesthesia and duration of anesthesia.

Unfortunately, only about one half of those to whom the questionnaire was sent made any reply, but practically all of those who did reply represent ophthalmolo-

gists of recognized ability and wide experience, so that a digest of their opinions as herewith given forms a valuable contribution to the subject.

ANESTHETIC PREFERRED

While a majority of those who answered the questionnaire admitted having tried many of the newer anesthetics, nearly all, or 98 per cent., now confine themselves to cocain, phenacain (holocain), and procain (novocain) in varying strengths, and each for specific purposes and with a specific reason for its use.

Practically all of the answers show that a distinction is made in the use to which the anesthetic is put. For instance, in cataract operations and all other operations in which the globe is opened, there is a very general preference for the local application of 4 per cent. cocain, though a few prefer 2 per cent. phenacain alone, still fewer the combination of 1 per cent. cocain with 2 per cent. phenacain, or 4 per cent. cocain in combination with 1 per cent. phenacain, and a very limited number (two) express a preference for 4 per cent. cocain, supplemented by the subconjunctival injection of 0.5 to 1 per cent. cocain.

In muscle operations about 25 per cent. of the answers show that the local application of from 4 to 5 per cent. solutions of cocain are relied on exclusively, though over 50 per cent. supplement this with either injections of procain (novocain) in from 1 to 2 per cent. solution, or cocain in 1 per cent. solution, and 5 per cent. of the answers indicate the use of pure powdered cocain over the operative field.

Those who use cocain for everything as well as those who use phenacain for everything are about equal in number, and few.

In glaucoma, or when using the tonometer, or in any case in which a dilated pupil is objectionable, phenacain is preferred by all but 5 per cent., and some of those who use cocain for everything employ a miotic to offset the effect of a dilated pupil when such is objectionable.

For foreign bodies in the cornea, and for surface anesthesia in connection with therapeusis, there is an overwhelming sentiment in favor of phenacain in from 1 to 2 per cent. solution.

In summarizing the answers to the first four questions in the questionnaire concerning the anesthetic preferred, strength used, and reasons for the preference, we find that the preponderance of evidence is in favor of a 4 per cent. solution of cocain as the local anesthetic for routine work. The reasons assigned for the preference are given as based on many years of experience, very satisfactory results from its use, and failure of other local anesthetics in one or more essentials to compare in reliability with cocain.

Practically all of the answers indicate that holocain in from 1 to 2 per cent. solution is used either frequently or occasionally in producing surface anesthesia for the removal of foreign bodies from the cornea, for local anesthesia in connection with therapeusis, for use prior to determining the tension with a tonometer, and for producing local anesthesia when a dilating effect on the pupil is to be avoided.

Local anesthesia with 4 per cent. cocain solution is supplemented with subconjunctival injections of a 1 per cent. cocain solution, to which a few drops of epinephrin solution have been added, in operations on the iris, muscles and lids. In sac operations, infiltration anesthesia with 1 per cent. procain (novocain) and epinephrin, or 1 per cent. cocain and epinephrin, shows opinion about equally divided. Two answers indicate a preference for infiltration anesthesia with 2 per cent. apothesin.

Three of the answers indicate that local application of 4 per cent. cocain, together with subconjunctival and deep orbital injections of 1 per cent. cocain and epinephrin, also is used for enucleation. However, a greater percentage of the men use procain (novocain) in from 1 to 2 per cent. solution by infiltration methods for operations on the muscles, lids and sac, and

enucleation, when general anesthesia is not to be employed.

OBJECTIONS TO NEWER ANESTHETICS

In condensed form, the objection made to all of the newer local anesthetics, as indicated by the answers, is that none of them present all of the advantages possessed by cocain, and many of them present distinct disadvantages, not the least of which is difficulty in procuring them. Alypin, beta-eucain, acoin and apothesin were mentioned in numerous answers, but with the single exception of one endorsement of apothesin for infiltration anesthesia for enucleation, all were condemned as unsatisfactory for ophthalmic work. Alypin was pronounced too feeble as well as too toxic, and the same objection was raised concerning beta-eucain. Acoin was considered by two as painful to use, and about 25 per cent. of the answers indicated that apothesin is next to useless as an instillation, and not so satisfactory as either cocain or procain (novocain) in infiltration anesthesia. It also is considered more toxic than procain (novocain), and though less toxic than cocain, is thought to be far less efficient than the latter agent in infiltration anesthesia. Even phenacain, which next to cocain is most in favor, offers the objection that it produces irritation of the eyes, must be prepared and kept in porcelain receptacles, and not infrequently is difficult to obtain.

STRENGTH OF SOLUTIONS PREFERRED

Concerning the strength of solutions, there is almost unanimity of opinion that a 4 per cent. solution of cocain and a 2 per cent. solution of phenacain will be proper concentrations for all but the surface anesthesia required for removal of foreign bodies from the cornea, applications of cautery or irritating remedies, or tonometry, when 1 per cent. solution of phenacain is considered sufficient. The combination of a 4 per cent. solution of cocain with a 1 per cent. solution of phenacain,

or a 1 per cent. solution of cocain with 2 per cent. solution of penacain for deep local anesthesia, and especially to effect anesthesia of the iris prior to cataract extraction, seems to be popular with several well known ophthalmologists of wide experience. For superficial or surface anesthesia one instillation of either cocain or phenacain solution is deemed sufficient, whereas for operative work four instillations at three minute intervals are deemed required. The use of stronger solutions as well as an increase of the number of instillations of the standard solutions, is thought to induce collapse of the cornea as well as delay in closure of the corneal wound in cataract extraction.

For infiltration anesthesia, a 1 per cent. solution of cocain is just as popular as a 2 per cent. solution of procain (novocain), even though the lessened toxicity of the latter is admitted. The addition of epinephrin not only adds to the efficiency of the solution by reducing the amount of hemorrhage during the operation; but through constriction of the blood vessels, it lessens the absorption of the anesthetic agent and hence reduces the possibility of toxic effects.

TIME OF ONSET AND DURATION OF ANESTHESIA

Five minutes after the instillation of either cocain or phenacain is considered in all of the replies as sufficient allowance for superficial or surface anesthesia, whereas fifteen minutes, or following immediately after the fourth instillation of either cocain or phenacain at three-minute intervals, is sufficient allowance for the deep anesthesia required for cataract extractions and other operations upon the eyeball. The anesthesia lasts from fifteen to twenty minutes, or long enough for the average ophthalmic operation.

In infiltration anesthesia, most operators allow from eight to ten minutes subsequent to the last injection to insure the maximum anesthesia, and they count upon holding the anesthesia for from thirty to thirty-five minutes.

IRRITATION AND VASCULARITY PRODUCED

All of the observers agree that local instillation of cocain in the eye allays irritation and reduces vascularity, whereas phenacain produces the opposite effect. Most of the ophthalmologists offset the congesting effect of phenacain by adding epinephrin to the solution.

EFFECT ON PUPILS, CORNEAL EPITHELIUM, ACCOMMODATION AND TENSION

Cocain dilates the pupils, desiccates and impairs the nutrition of the cornea, and produces a slight paresis of accommodation, whereas phenacain has no such effects.

Neither cocain nor phenacain affects the tension in the normal eye, but the tension of the glaucomatous eye may be increased by the effect of the dilation of the pupil produced by cocain.

TOXIC EFFECTS

Owing to the fact that only small amounts of local anesthetics are used in ophthalmic practice, the occurrence of toxic effects is rare. However, about 10 per cent. of the answers to our questionnaire mentioned having seen mild toxic disturbances from both instillations and injections of cocain, and in three instances rather alarming symptoms were produced. It is but fair to report that the case presenting the most marked symptoms of collapse occurred following infiltration rather than instillation of a cocain solution, with consequent greater absorption and greater dose of the drug.

Some observers are inclined to believe that the mild toxic symptoms seen after instillation of solutions of cocain may be due to psychic influence as much as to the toxic effects of the cocain.

To offset the theory that toxic effects are not secured from instillations of 4 per cent. solution of cocain in the eye, your committee desires to call attention to an interesting feature in connection with this question,

which is that in all of the answers to our questionnaire there is not a report of a case of toxic disturbance from phenacain. This also leads us to question the statement often made that phenacain is more toxic than cocain.

The toxic symptoms observed and described are at first a sense of exhilaration, followed by giddiness, oppressed breathing, pallor, nausea, clammy perspiring skin and general weakness. All of these symptoms usually disappear promptly after the patient lowers the head even with the knees, or is placed in a reclining position.

STABILITY OF SOLUTIONS

Your committee has been interested in the conflicting reports concerning the stability of solutions of cocain, especially when sterilized by boiling. Fully 75 per cent. of those who answered the questionnaire reported that boiling does not impair the efficiency of any of the agents used for the production of local anesthesia, though three well-known members of this section, of ability and wide experience, assert that cocain solutions deteriorate through boiling. To enable the committee to arrive at more definite conclusions concerning this question, several clinicians were asked to compare the effects of boiled and unboiled solutions of cocain of the same strength in individual cases as well as in a series of clinical cases, and the returned reports indicate no difference in the anesthetic effect produced by the solutions that were sterilized by boiling and those not subjected to heat. The question arises, therefore, as to the possibility of erroneous impressions being formed as a direct result of insufficient clinical investigation.

Many of those who answered the questionnaire reported that they invariably sterilize their cocain solutions by boiling a few minutes prior to use in operative work, while others content themselves with dissolving the cocain crystals in water that already has been sterilized by boiling. All of the replies indicate that nothing but freshly prepared solutions of cocain are used in operative work. Solutions will remain

active for a much longer time if 5 grains of boric acid is added to each ounce of the solution. One correspondent recommends the addition of 10 grains of chlorbutanol to each ounce of cocain solution, and asserts that it not only preserves the solution for several weeks but intensifies the anesthesia.

EFFECT OF ADDITION OF EPINEPHRIN OR OTHER AGENTS ON THE INTENSITY AND DURATION OF ANESTHESIA

Fully 80 per cent. of those answering the questionnaire asserted that epinephrin added to phenacain and procain (novocain) solutions intensifies as well as slightly prolongs the anesthetic effect. However, concerning this subject, we desire to quote from the pharmacologic studies on local anesthesia conducted by Sollmann, and reported in THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, Jan. 26, 1918, p. 216, in which it is stated that the addition of an alkali to the local anesthetics other than phenacain, increases their anesthetic efficiency, as tested on the motor as well as the sensory fibers of nerve trunks, and increases the action of the anesthetics when applied to the surface as on the cornea. This increased activity by an alkali is due to the liberation of the anesthetic base which penetrates more readily than the salt. No increased activity occurs through the addition of alkalis when the anesthetic is used by infiltration.

Sollmann further states that the addition of an equal volume of 0.5 per cent. solution of sodium bicarbonate to a solution of cocain for surface anesthesia increases the activity of the cocain from one to two times; the activity of beta-eucain is increased two times; procain from two to four times; tropacocain and alypin, four times. This effects a marked saving in the amount of anesthetic drug used.

The efficiency of phenacain is destroyed by the addition of an alkali.

COMBINATION OF ANESTHETICS

Inasmuch as some of the answers to our questionnaire indicated preference for various combinations of anesthetics, especially a combination of phenacain and cocain, we desire to call attention to the results of animal experimentation and study of the subject by Sollmann, THE JOURNAL A. M. A., Jan. 26, 1918, p. 218, from which we quote:

> The efficiency of mixtures of the local anesthetics corresponds to more or less complete summation without any potentiation. In other words, if two solutions of different anesthetics are diluted until they are "just effective," then a mixture of the two solutions will also be "just effective," no more and no less.

While nothing is gained by combination of anesthetics from the standpoint of efficiency, the undesirable side effects perhaps may be minimized.

RELATIVE TOXICITY: ANIMAL EXPERIMENTATION

The occurrence and symptoms of acute cocain poisoning are so well known that it is unnecessary to do more than call attention to the fact. So far as we know, the literature contains no record of fatal results from cocain poisoning when the drug has been used purely for ophthalmic work, though numerous observers report slightly or even moderately severe toxic symptoms from instillations of even a 4 per cent. solution of cocain in the eye. It may be argued that psychic influences play a prominent rôle in these cases, though one must not forget the possibility of an extremely well-marked idiosyncrasy.

Concerning the relative toxicity and range of concentration of solutions for the several local anesthetics, attention may be called to the various investigations reported by Eggleston and Hatcher, in the *Journal of Pharmacology and Experimental Therapeutics,* August, 1919, p. 433, and in THE JOURNAL A. M. A., Oct. 25, 1919, p. 1256, a digest of which is herewith presented.

The search for substitutes for cocain has led to the introduction into materia medica of a large number of compounds possessing the property of causing local anesthesia, and for each of these the claim has been made that it is less toxic than cocain. These compounds belong to several general chemical groups, and not only do these compounds of somewhat diverse chemical nature possess the common property of producing local anesthesia, but also they resemble one another very closely in all their more important pharmacologic actions, such differences as are shown being chiefly quantitative. All are capable of producing severe or even fatal acute poisoning in man, with symptoms closely related to those produced by cocain.

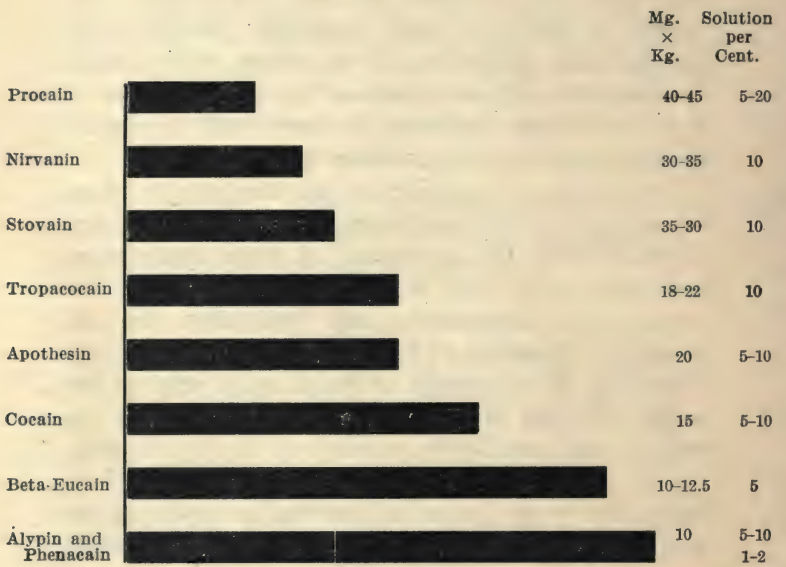
The rapidity of absorption of the drug into the blood stream seems to be the feature of greatest importance in determining the occurrence of acute intoxication, especially in the case of the several substitutes for cocain. An investigation was undertaken by the Research Committee of the Council on Pharmacy and Chemistry in an attempt to throw some light on the causes of acute intoxication in man produced by the several local anesthetics, to discover if possible some of the factors which influence the toxicity of these drugs, and to find means of avoiding or combating the acute toxic actions. More than 300 animal experiments were performed, all of which were made on cats, which more closely resemble man in their response to most drugs than do the rodents. The local anesthetics employed were alypin, apothesin, beta-eucain, cocain, phenacain, nirvanin, procain (novocain), stovain and tropacocain.

The relative toxicity of these local anesthetics is shown in the accompanying chart.

From the chart it will be seen that cocain occupies an intermediate position with reference to its maximal toxicity, being surpassed by alypin, beta-eucain and phenacain, while the least toxic are nirvanin and pro-

cain. It will be noted that apothesin is but slightly less toxic than cocain, and is a little more than twice as toxic as procain (novocain).

It also was shown that many times the minimum fatal dose of procain (novocain) can be injected intravenously *if the injection is made slowly*. The recovery from a sublethal dose is very rapid except in the case of cocain, even when the dose approaches closely to the



Relative toxicity, fatal doses in milligrams per kilogram, and range of concentration of solutions for the several local anesthetics.

fatal. Cocain cannot be given by repeated intravenous injection of small doses without the development of evidences of accumulation, as shown by the increasing severity of the symptoms following the later repetitions, and by the ultimate death of the animal after five times the minimum single dose given in a period of four hours and sixteen minutes. In the case of procain (novocain), the simultaneous administration of epinephrin greatly reduces the toxicity.

The elimination of cocain and phenacain is a slow process, and in the case of cocain it may not be com-

plete after periods of two or more days. Cocain and phenacain are relatively slowly destroyed, so that even when absorption is delayed by the vasoconstrictor effect of epinephrin they are still capable of accumulation in the essential organs sufficient to cause serious intoxication or death when from five to six times the fatal vein dose is injected subcutaneously.

On the other hand, the importance of the rapid destruction of the other members of the group is emphasized by the experiments with epinephrin, and it would seem to point to the decided value of always using epinephrin as a routine in combination with the cocain substitutes for subcutaneous injection in man, to delay absorption and diminish the chances of accidental poisoning.

Eggleston and Hatcher again state, THE JOURNAL A. M. A., Oct. 25, 1919, p. 1258, that in order to diminish the likelihood of intoxication from the subcutaneous injection of the local anesthetics excepting cocain and phenacain, epinephrin should be added to the solution as a routine, because by delaying their absorption it renders it probable that the destruction by the liver can keep the amount present in the circulation at any time at a point below that sufficient to cause intoxication. The use of epinephrin also has the further advantages of prolonging the anesthetic action of a given quantity of the drug and reducing the amount required to remain in contact with the tissue, and by maintaining the contact for a longer period of time than when the drug is injected alone.

LITERATURE

While your committee has reviewed a great deal of literature pertaining to local anesthesia in ophthalmic work, most of which is the expression of individual opinions, it does not seem necessary to quote extensively or give the large number of references that would be required in order to do the subject justice. For the most part, the opinions expressed in recent literature

are not at variance with the deductions drawn by your committee from an analysis of the experience of our members, and the results of experimental investigation carried on by the Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association. We especially are impressed with the favor shown infiltration anesthesia in ophthalmic work by so many of the prominent European ophthalmologists of wide clinical experience.

Perhaps the latest and most comprehensive work is that entitled, "L'Anesthesie locale en ophthalmologie", by C. Duverger, professor of clinical ophthalmology at Strasbourg. (Translated for the Committee by Dr. Pierre Gaudissart, Brussels.) A résumé of the principal points contained in that work is given herewith:

I. Anatomic Researches

Conclusions drawn from experiments on skull and on cadaver.

(*a*) Needle 4.5 cm., reserved for total anesthesia of the superior maxillary nerve, or when there is edema of the region.

(*b*) Needle 3.5 cm., suffices in all other cases and can never injure the optic nerve.

(*c*) Needle 2.5 c., for skin, conjunctiva and lacrimal sac.

II. Solutions

(*a*) For instillations in culdesac, cocain from 5 to 10 per cent solutions.

(*b*) For injections:

(1) Cocain solution 1 per cent. at the most, and better 0.5 per cent.

(2) Procain (novocain) solution is much better (Anesthetic power three times greater than cocain; ten times less toxic.)

In general surgery, 1 gm. may be used. For the eye, on account of the strong absorption power of orbital tissues, it is better never to inject more than 50 centigrams.

Concentration.—Four per cent. solution is the best.

Anesthesia of the whole content of the orbit requires only four injections of 2 c.c. temperature 32 C.

For broad plastics of the face, injections of from 1 per cent. to 0.5 per cent. dilutions are the best.

Epinephrin is useful in weak concentrations:

Solution 1

Procain .....0.20 gm.
Epinephrin .....(1/1,000) 2 drops
Water .....5 c.c.

Solution 2

Procain .....0.10 gm.
Epinephrin .....(1/100) 4 drops
Water .....10 c.c.

General Methods

Local anesthesia (instillations, infiltrations of tissues.)

Regional anesthesia; anesthesia of nervous branches.

Infiltration anesthesia is inferior to regional anesthesia because it alters the field by swelling and does not succeed with scar tissue and inflamed tissue.

Instillations must precede every operation. They reach superficial parts of the conjunctiva, cornea and iris. They never reach deep layers of the conjunctiva, subconjunctival tissues or ciliary body, and they are without action on inflamed tissues.

Anesthesia of the Globe

1. *Cornea.*—When the eye is noninflamed, instillation. When the eye is inflamed, instillation only suffices for extraction of foreign body, cauterization or tonometry.

If there is traction on the conjunctiva, or pressure on the globe or emptiness of anterior chamber, conjunctival or deep anesthesia is required.

2. *Conjunctiva (Bulbar).*—The zone next to the limbus, breadth of 3 mm., innervated by the ciliary nerves, is anesthetized by the deep injection for anesthesia of the globe.

With all the operations which require a more extensive insensibility, subconjunctival infiltration, with a small and acute needle, and the 2 or 4 per cent. procain solution, must be used. This anesthesia also must be made in every complete anesthesia of the globe, for instance, in enucleation.

3. *Anterior Chamber.*—Cysts or Iridectomy: When the eye is white, instillations of from 5 to 10 per cent. cocain solution. When the eye is red and inflamed; deep anesthesia of the globe is required.

4. *Deep Anesthesia of the Globe.*—With 3.5 cm. needle, the author never has had the slightest accident. From 2 to 3 c.c. of 4 per cent. procain solution.

Injection: Lower and outer angle of rim of orbit. Needle is thrust in just against the bone, guided by the tip of the finger. Needle directed backward, upward and slightly inward, aiming to hug the floor of the orbit, then pushed in until its base reaches the orbital margin. During this time 0.5 c.c. is

injected. The 1.5 c.c. remaining must be injected at several intervals, each time changing the position of the point of the needle. In that way, all the nervous branches of the muscular cone are reached by the diffusion of the liquid. In very inflamed eyes, it is better to inject 3 c.c. instead of 2 c.c.

As a second step, the subconjunctival infiltration must be done, but *only as a second step.*

The advantage of the injection through the skin is that in this way the painful globe is not touched.[1]

5. *Lids and Palpebral Conjunctiva.*—Anesthesia of the four nervous branches (nasal, frontal, lacrimal and suborbital) is too complicated when an operation is on the lids only.

The following method is more simple: From 2 to 4 per cent. solution is used. A needle, 2.5 cm., is introduced, 0.5 cm. to the outer side of the external canthus and 0.5 cm. below the external canthus. It is conducted midway between the skin and conjunctiva up to the inner canthus. When both lids are concerned, anesthesia may be obtained by the same puncture, by tilting the needle. This injection reaches all the nervous fibers which run from the base toward the margin of the lid. This injection suffices for all the operations on the lids.

For the Motais operation:

(*a*) Anesthesia of the upper lid.

(*b*) Needle thrust a second time exactly at the middle of the orbital margin, backward, parallel to the roof of the orbit, 2.5 cm. needle, 1 c.c. injected.

6. *Palpebral Plastics.*—Preliminary anesthesia of the lids is required.

(*A*) Autoplasty with Frontal Flap: Four and five-tenths cm. needle is thrust in at outer end of eyebrow up to the periosteum and pushed in, keeping in contact with the bone to the inner end of the eyebrow. During this time the liquid is injected slowly: 2 c.c. of a 4 per cent. solution.

The first step is common to all the frontal plastics.

The second varies.

(*a*) Flap with internal pedicle.

Needle thrust at inner angle of eyebrow of the other side, then pushed vertically in the median line up to the limit between upper third and inferior two thirds. All the branches coming from the opposite side are reached in that way.

(*b*) Flap with external pedicle.

Needle thrust near outer canthus and pushed backward, more or less upward, according to the situation of the pedicle.

(*B*) Autoplasty with Temporal Flap:

(*a*) Injection on the outer third of the orbital margin.

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1. For the Elliot operation, when the eye is quiet, repeated instillations and subconjunctival infiltration in the upper part are sufficient.

(*b*) Anesthesia of the frontolacrimal nerve in the sphenoidal cleft.

(*C*) Autoplasty with Maxillary Flap:

(*a*) Anesthesia of the suborbital nerve.

Patient clenches teeth, with the operator's finger on the point where masseter muscle begins to be inserted on bone. On the line connecting this point to the edge which marks the upper part of the wing of the nose, the needle is thrust a trifle inside the middle of this line, and pushed upward, backward and outward so that its direction cuts the middle of the mouth and that its angle with the tissues is 20 degrees (practically the thickness of the finger). One c.c. of 6 per cent. solution is the anesthetic for the lower lid, the inner part of the cheek and the wing of the nose.

(*b*) Lacrimal.

(*c*) Inferior and outer barrier.

Needle thrust in the cheek, a trifle above external angle of lips, pushed backward up to the vertical branch of the lower jaw, then thrust again and pushed upward to the level of the tragus of the ear. From 5 to 6 c.c. of 2 per cent. solution is used.

7. *Muscles-Strabismus.*—Deep injection as used for the complete anesthesia of the globe.

8. *Orbital Anesthesia.*—(*a*) Anesthesia of the nasal nerve.

The finger is placed on the pulley of the superior oblique muscle, a needle, 3.5 cm., is thrust against the inner side of the pulley, slips against the orbital wall backward, always with the point kept in contact with the supero-internal angle, with the base of the needle against the margin 0.5 c.c. of a 4 per cent. solution is injected. Thus anesthesia of all the branches of the nasal nerve is obtained with the roots of the ophthalmic ganglion.

(*a*) Anesthesia of frontal and lacrimal nerves.

The needle is thrust in at the outer angle of the margin of the orbit, above the palpebral ligament. It is pushed carefully horizontally, with care not to break the needle against the bone. Five-tenths c.c. of a 4 per cent. solution is injected when the base of the needle is against the margin.

(*c*) Anesthesia of superior maxillary nerve: in spheno-maxillary fissure.

A needle 4.5 cm. is thrust against the orbital margin, the outer and lower angle being 10 or 12 mm. inside this angle. It is pushed in the anteroposterior plane, backward and downward. When the base is against the margin, the injections are made.

CONCLUSIONS

In analyzing the results of this investigation, so far as our present knowledge of ophthalmic work is con-

cerned, your committee feels justified in arriving at the following conclusions:

1. For surface anesthesia, cocain in 4 per cent. solution, freshly made, possesses distinct advantages over all other local anesthetics, particularly for operative work.

Concerning cocain anesthesia the following is offered:

(*a*) In all instances, the anesthesia is equal to and in most cases it is greater than that produced by any other local anesthetic.

(*b*) Its toxicity when used in the small dosage required for ocular anesthesia is almost negligible and does not count as a serious objection.

(*c*) The desiccation and disturbance of nutrition of the cornea produced by it are negligible or entirely avoided if care is observed in keeping the eyelids closed after the instillations of the cocain solution and up to the time that the operative work is to begin.

(*d*) The dilatation produced by the cocain is of short duration, does not often occasion inconvenience, and may be overcome promptly by the counter effect of a weak miotic.

(*e*) The penetrating effect of cocain solution is increased by the addition of 0.5 per cent. solution of sodium bicarbonate.

(*f*) The efficiency of cocain solution is not impaired by boiling.

(*g*) The efficiency of cocain solutions is not affected either as to intensity or prolongation of anesthesia by the addition of epinephrin.

(*h*) The use of stronger solutions than the one recommended are at the risk of seriously disturbing the nutrition of the cornea and interfering with the healing process.

2. Phenacain in 2 per cent. solution stands next to cocain in efficiency.

Concerning phenacain anesthesia the following is offered:

(*a*) It has the advantage of producing a quicker effect than cocain and a slight antiseptic action.

(*b*) It does not dilate the pupil, hence is valuable in producing surface anesthesia for tonometry, therapeusis and removal of foreign bodies from the cornea.

(*c*) It does not produce desiccation of the cornea, nor, so far as known, disturb nutrition.

(*d*) The solutions are not affected by boiling.

(*e*) Epinephrin does not add to its efficiency in any way.

(*f*) Alkalis should not be added to phenacain solutions as they cause precipitation.

(*g*) Phenacain offers the distinct disadvantage of producing more or less irritation, which is very objectionable to sensitive patients.

(*h*) Phenacain is incompatible with alkalis and their carbonate bases, and the use of glass vessels should be avoided in preparing the solution, porcelain being used instead.

3. Procain (novocain) in 2 per cent. solution is the anesthetic of choice for infiltration anesthesia.

(*a*) The addition of epinephrin does not increase efficiency, but does delay absorption and diminish the chances of accidental poisoning.

(*b*) Procain (novocain) solutions should be injected slowly to aid in the avoidance of toxic effects.

(*c*) The efficiency of procain (novocain) solutions is not increased by the addition of alkalis.

RECOMMENDATIONS

Your committee believes that it is highly advisable to find, if possible, a synthetic anesthetic which will take the place of cocain—one that will be less toxic, less expensive, nonhabit-forming and equally efficient.

The Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association is now experimenting with several new local anesthetics not yet on the market, one of which is especially promising, though it must be tested clinically

before expectations are raised. Your committee has been placed in possession of samples of this promising local anesthetic but has not had time to conduct sufficient tests to warrant an expression of opinion. The results of experiments in progress seem to indicate that cocain may be replaced successfully by one or more new synthetic anesthetics, although no definite announcement concerning the matter will be made until after adequate clinical tests justify some announcement. To that end we recommend that a committee be selected to cooperate with the Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association in carrying out such clinical investigations and experimental study, under suitable checks as to methods and technic as may seem indicated, in order to determine the advantages and disadvantages of the newer local anesthetics as they may be offered, as well as to continue the further study of any of the local anesthetics at present under study or that are considered in this report.

Your committee also recommends the further study of the advantages and disadvantages of infiltration anesthesia in ophthalmic work, with the possibility in view of recommending a more general employment of such form of anesthesia in ophthalmic operative work. Such an investigation should embrace a study of the agents adaptable to the purpose, the strength of the solutions and technic of application.

Any and all investigations should be independent of and uninfluenced by the recommendations or reports of manufacturers who usually are prejudiced because of the commercial interest in view.

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